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(54) Title: COMPOSITIONS AND METHODS USING SAME FOR TREATING AMYLOID-ASSOCIATED DISEASES

(57) Abstract: Compounds having one or more phenol moieties, novel derivatives thereof, compositions containing same and uses thereof for the treatment of amyloid-associated diseases are provided.

COMPOSITIONS AND METHODS USING SAME FOR TREATING AMYLOID-ASSOCIATED DISEASES

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to compounds, compositions containing same and methods using same for preventing amyloid fibril formation. More particularly, the present invention relates to the use of polyaromatic compounds such as phenol red and novel derivatives thereof for the treatment of amyloid-associated diseases.

Amyloid material deposition (also referred to as amyloid plaque formation) is a central feature of a variety of unrelated pathological conditions including Alzheimer's disease, prion-related encephalopathies, type II diabetes mellitus, familial amyloidosis and light-chain amyloidosis.

Amyloid material is composed of a dense network of rigid, nonbranching proteinaceous fibrils of indefinite length that are about 80 to 100 Å in diameter. Amyloid fibrils contain a core structure of polypeptide chains arranged in antiparallel β-pleated sheets lying with their long axes perpendicular to the long axis of the fibril [Both et al. (1997) Nature 385:787-93; Glenner (1980) N. Eng. J. Med. 302:1283-92].

Approximately twenty amyloid fibril proteins have been identified in-vivo and correlated with specific diseases. These proteins share little or no amino acid sequence homology, however the core structure of the amyloid fibrils is essentially the same. This common core structure of amyloid fibrils and the presence of common substances in amyloid deposits suggest that data characterizing a particular form of amyloid material may also be relevant to other forms of amyloid material and thus can be implemented in template design for the development of drugs against amyloid-associated diseases such as type II diabetes mellitus, Alzheimer's dementia, or prion-related encephalopathies.

Furthermore, amyloid deposits do not appear to be inert in vivo, but rather are in a dynamic state of turnover and can even regress if the formation of fibrils is halted [Gillmore et al. (1997) Br. J. Haematol. 99:245-56].

Thus, therapies designed to inhibiting the production of amyloid polypeptides or inhibiting amyloidosis may be useful for treating amyloid associated diseases.

Inhibition of amyloid polypeptides production - Direct inhibition of the production of amyloid polypeptides may be accomplished, for example, through the use of antisense oligonucleotides such as against human islet amyloid polypeptide

messenger RNA (mRNA). In vitro, the addition of antisense oligonucleotides or the expression of antisense complementary DNA against islet amyloid polypeptide mRNA increased the insulin mRNA and protein content of cells, demonstrating the potential effectiveness of this approach [Kulkarni et al. (1996) J. Endocrinol. 151:341-8; Novials et al. (1998) Pancreas 17:182-6]. However, no experimental results demonstrating the *in vivo* effectiveness of such antisense molecules have been demonstrated.

Inhibition of amyloid fibril formation - Amyloid, including islet amyloid, contains potential stabilizing or protective substances, such as serum amyloid P component, apolipoprotein E, and perlecan. Blocking their binding to developing amyloid fibrils could inhibit amyloidogenesis [Kahn et al. (1999) Diabetes 48:241-53], as could treatment with antibodies specific for certain parts of an amyloidogenic protein [Solomon et al. (1997) Proc. Natl. Acad. Sci. USA 94:4109-12].

The following summarizes current attempts to engineer drugs having the capability of destabilizing amyloid structures.

Destabilizing compounds - Heparin sulfate has been identified as a component of all amyloids and has also been implicated in the earliest stages of inflammation-associated amyloid induction. Kisilevsky and co-workers (Nature Med. 1:143-148, 1995) described the use of low molecular weight anionic sulfonate or sulfate compounds that interfere with the interaction of heparin sulfate with the inflammation-associated amyloid precursor and the β peptide of Alzheimer's disease (AD). Heparin sulfate specifically influences the soluble amyloid precursor (SAA2) to adopt an increased β-sheet structure characteristic of the protein-folding pattern of amyloids. These anionic sulfonate or sulfate compounds were shown to inhibit heparin accelerated $A\beta$ fibril formation and were able to disassemble preformed fibrils in vitro, as monitored by electron micrography. Moreover, these compounds substantially arrested murine splenic inflammation-associated amyloid progression in vivo in acute and chronic models. However, the most potent compound [i.e., poly-(vinylsulfonate)] showed acute toxicity. Similar toxicity has been observed with another compound, IDOX (Anthracycline 4'-iodo-4'-deoxy-doxorubicin), which has been observed to induce amyloid resorption in patients with immunoglobin light chain amyloidosis (AL) [Merlini et al. (1995) Proc. Natl. Acad. Sci. USA].

Destabilizing antibodies - Anti-β-amyloid monoclonal antibodies have been shown to be effective in disaggregating β-amyloid plaques and preventing β-amyloid plaque formation *in vitro* (U.S. Pat. No. 5,688,561). However, no experimental results demonstrating the *in vivo* effectiveness of such antibodies have been demonstrated.

Small molecules – The potential use of small molecules which bind the amyloid polypeptide and stabilizing the native fold of the protein has been attempted in the case of the transthyretin (TTR) protein [Peterson (1998) Proc. Natl. Acad. Sci. USA 95:12965-12960; Oza (1999) Bioorg. Med. Chem. Lett. 9:1-6]. Thus far, it has been demonstrated that molecules such as thyroxine and flufenamic acid are capable of preventing the conformation change, leading to amyloid formation. However, the use of the compounds in animal models has not been proved yet and might be compromised due to the presence in blood or proteins, other than TTR, capable of binding these ligands.

Antioxidants - Another proposed therapy has been the intake of antioxidants in order to avoid oxidative stress and maintain amyloid proteins in their reduced state (i.e., monomers and dimers). The use of sulfite was shown to lead to more stable monomers of the TTR both in vitro and in vivo [Altland (1999) Neurogenetics 2:183-188]. However, a complete characterization of the antioxidant effect is still not available and the interpretation of results concerning possible therapeutic strategies remains difficult.

Destabilizing peptides - The finding that the addition of synthetic peptides that disrupt the ß-pleated sheets ("ß-sheet breakers") dissociated fibrils and prevented amyloidosis [Soto et al. (1998) Nat. Med. 4:822-826] is particularly promising from a clinical point of view. In brief, a penta-residue peptide inhibited amyloid beta-protein fibrillogenesis, disassembled preformed fibrils in vitro and prevents neuronal death induced by fibrils in cell culture. In addition, the beta-sheet breaker peptide significantly reduced amyloid beta-protein deposition in vivo and completely blocked the formation of amyloid fibrils in a rat brain model of amyloidosis.

Green tea extracts – U.S. Patent Applications having the Publication Nos. 20020086067 and 20020151506 teach the use of various components of green tea extracts for treating an amyloid disease. While these patent applications teach that these components inhibit amyloid fibril formation, they fail to teach neither a

mechanism nor a common structural feature which provides these green tea components with such an activity.

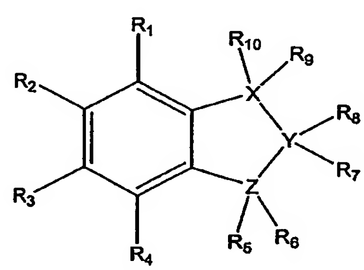
The present inventors have previously shown that aromatic interactions play a key role in amyloid fibril formation by serving as structural and functional elements that direct molecular recognition and self-assembly.

While conceiving the present invention, it was therefore envisioned that compounds having a plurality (e.g., two or more) of aromatic moieties, which may participate in such aromatic interactions, could efficiently serve as inhibitors of amyloid fibril formation.

While reducing the present invention to practice, the present inventors indeed uncovered that amyloid formation can be strongly inhibited by polyaromatic compounds, such as phenol red (PR), suggesting use of these compounds in the treatment of amyloid-associated diseases.

SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided use of a compound having the general Formula I:



Formula I

a pharmaceutically acceptable salt thereof or a prodrug thereof,

wherein:

X, Y and Z are each independently selected from the group consisting of carbon, oxygen, sulfur, $CR_{11}R_{12}$ or $R_{13}R_{14}C$-$CR_{15}R_{16}$, provided that at least one of X, Y and Z is oxygen or sulfur;

R_1-R_{16} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, alkoxyphenyl,

thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1-R_4 and/or at least two of R_5-R_{16} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

whereas:

at least one of R_1-R_4 is selected from the group consisting of hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, O-carboxy and O-thiocarboxy and/or at least one of R_5-R_{16} comprises phenol, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl hydroxyphenol, and dihydroxyphenol,

for the manufacture of a medicament identified for the treatment of amyloid-associated diseases.

According to further features in preferred embodiments of the invention described below, X is carbon; Y is $R_{13}R_{14}C$-$CR_{15}R_{16}$; and Z is oxygen.

According to still further features in the described preferred embodiments R_9 is oxo; and R_{10} is absent.

According to still further features in the described preferred embodiments at least one of R_{13}-R_{16} is selected from the group consisting of alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol and dihydroxyphenol.

According to still further features in the described preferred embodiments each of R_1 and R_3 is hydroxy.

According to still further features in the described preferred embodiments at least one of R_{13}-R_{16} is alkyl.

According to still further features in the described preferred embodiments, X is carbon; Y is oxygen; Z is carbon or sulfur; and at least one of R_5 and R_6 is oxo.

According to still further features in the described preferred embodiments at least one of R_9 and R_{10} is selected from the group consisting of alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol and dihydroxyphenol.

According to still further features in the described preferred embodiments the compound is selected from the group consisting of phenol red, dimethoxy phenol red, methoxy phenol red, diacetoxy phenol red, acetoxy phenol red, pyrocatechol violet, phenolphthalein, catechin, epigallocatechin gallate, epicatechin gallate, epicatechin, epigallocatechin, eriodictyol, quercetin, procyanidin, hydroxyphenyl, tocopherol and bromophenol red.

According to another aspect of the present invention there is provided an article-of-manufacture comprising a packaging material and a pharmaceutical composition identified for treating amyloid-associated diseases being contained within the packaging material, the pharmaceutical composition including, as an active ingredient, the compound described hereinabove, and a pharmaceutically acceptable carrier.

According to yet another aspect of the present invention there is provided a method of treating an amyloid-associated disease in a subject, the method comprising administering to a subject in need thereof, a therapeutically effective amount of the compounds described hereinabove, thereby treating the amyloid-associated disease in the subject.

According to further features in preferred embodiments of the invention described below, the administering is effected at a concentration of the compound not exceeding 4mg/Kg body weight/hour.

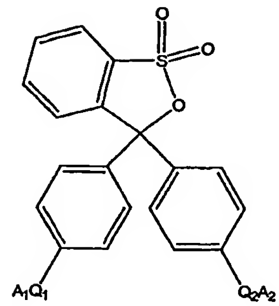
According to still further features in the described preferred embodiments the administering is effected orally.

According to still another aspect of the present invention there is provided a pharmaceutical composition comprising a therapeutically effective amount of the compound described hereinabove and a pharmaceutically acceptable carrier.

According to still further features in the described preferred embodiments the pharmaceutical composition further comprising an anti-amyloid drug.

According to still further features in the described preferred embodiments the anti-amyloid drug is selected from the group consisting of an amyloid-destabilizing antibody, an amyloid-destabilizing peptide and an anti-amyloid small molecule.

According to still another aspect of the present invention there is provided a novel compound having the general Formula II:



Formula II

a pharmaceutically acceptable salt thereof or a prodrug thereof,

wherein Q_1 and Q_2 are each independently selected from the group consisting of oxygen and sulfur; and

A_1 and A_2 are each independently selected from the group consisting of hydrogen, alkyl, aryl, cycloalkyl and carbonyl,

whereas when Q_1 and Q_2 are each oxygen, one of A_1 and A_2 is hydrogen and the other is selected from the group consisting of alkyl, cycloalkyl, aryl and carbonyl, preferably methyl or acetyl.

The present invention successfully addresses the shortcomings of the presently known configurations by providing compositions and methods using same for preventing amyloid fibril formation.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and

readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIGs. 1a-b are graphs depicting inhibition of hIAPP core amyloidogenic peptide ($hIAPP_{22-29}$ and $hIAPP_{20-29}$) aggregation in the presence of phenol red. 1 mM peptide was dissolved in Tris buffer (pH 7.2) and 4% DMSO. Phenol red inhibitor was dissolved in the same buffer conditions to a final concentration of 10 mM. For each sample turbidity was measured continuously at 560 nm. Figure 1a - $hIAPP_{22-29}$ (NFGAILSS) aggregation. Figure 1b - $hIAPP_{20-29}$ (SNNFGAILSS) aggregation with phenolphthaleine as control. Background values of the buffer and phenol red were reduced from the relevant measurements.

FIGs. 2a-b are graphs depicting secondary structure transition of $hIAPP_{1-37}$ peptide in the absence (Figure 2a) or presence (Figure 2b) of phenol red, as measured using Circular Dichroism. Human $hIAPP_{1-37}$ was dissolved in HFIP and diluted into sodium acetate buffer (pH 6.5) to a final concentration of 4 μM and 1% HFIP with or without 40 μM phenol red. Non-soluble peptide was removed using centrifugation. Note that in the absence of inhibitor (Figure 2a), $hIAPP_{1-37}$ shows a transition from random coil conformation within 6 hours, and this transition reaches a maximal value within 26 hours. However, in the presence of 40 μM phenol red inhibitor (Figure 2b) there is an evident inhibition of transition to β sheet conformation (218 nm peak).

FIGs. 3a-c is a graph depicting the effect of phenol red on human $IAPP_{1-37}$ fibril formation, as determined by Thioflavin T fluorescence assay. Figure 3a - Human $IAPP_{1-37}$ was dissolved in HFIP and diluted into sodium acetate buffer (pH 6.5) to a final concentration of 4 μM and 1% HFIP with or without 40 μM phenol red. Non-soluble peptide was separated using centrifugation. Fluorescence values were measured after the addition of 3 μM Tht to each sample. Note, following a lag phase of approximately 20 hours the fluorescence values increased significantly without inhibitor in contrast to constant low levels in the presence of the phenol red inhibitor. Figure 3b – As in Figure 3a only gradual concentration of phenol red was used along with a more sensitive fluorometer which enabled the dilution of the sample 10 folds

so that maximal phenol red in the measured sample did not exceed 4μM. As shown a dose dependent inhibition was evident with ~ IC50 of 2.5 μM. Figure 3c – Shows end point fluorescence of the samples of Figure 3b following 5 days of incubation.

FIGs. 4a-c are photomicrographs depicting the morphology of hIAPP$_{22\text{-}29}$, hIAPP$_{20\text{-}29}$ peptides, and hIAPP$_{1\text{-}37}$ polypeptide in the absence or presence of phenol red and phenolphthaleine inhibitors. hIAPP$_{22\text{-}29}$ and hIAPP$_{20\text{-}29}$ peptides were prepared as described in Figures 1a-c and hIAPP$_{1\text{-}37}$ as in Figures 3a-c. Samples were generated by staining 10 μl sample with Uranyl acetate and then viewed with a JEOL 1200EX electron microscope operating at 80 kV. Distinct morphological differences are evident for all peptides, which showed a kinetic inhibitory effect of fibril formation by phenol red. In contrary, no inhibition effect was evident by phenolephthaleine molecule (Figure 4b).

FIGs. 5a-b are histograms depicting inhibition of hIAPP fibril formation by green tea polyphenols, as determined by Tht fluorescence assay. Color code for Figure 5a: Blue – 48 hr., red 3 days, yellow – 8 days, green 12 - days. Note, detailed observation of the inhibitory effect following 3 days of incubation in the presence of green tea polyphenols (Figure 5b) revealed high efficacy for the gallate group.

FIG. 6 presents comparative plots demonstrating the inhibition of hIAPP$_{1\text{-}37}$ fibril formation by pyrocatechol violet, as determined by ThT fluorescence assay (squares denote no addition of inhibitor, triangles denote addition of 4 μM pyrocatechol violet and circles denote addition of 40 μM pyrocatechol violet).

FIG. 7 presents the chemical structures (2D) of the inhibitors phenol red, pyrocatechol violet, phenolphthaleine, Diacetoxy phenol red (PF3) and Dimethoxyphenol red (PF4).

FIGs. 8a-b are histograms depicting inhibition of hIAPP$_{1\text{-}37}$ fibril formation by PF3 and PF4 as determined by Thioflavin T fluorescence assay. Samples were either centrifuged immediately after dilution of hIAPP stock solution (Figure 8a) or left uncentrifuged (Figure 8b).

FIG. 9 is a bar graph showing a dose dependent rescue effect of phenol red on PC12 cells incubated in the presence of hIAPP$_{1\text{-}37}$. Values are mean±SD (n=4).

FIGs. 10a-h show the rescue effect of PR on pancreatic β-cells incubated in the presence of hIAPP aggregates. βTC-tet rodent β-cells were incubated for 24 hrs with 4 μM hIAPP$_{1\text{-}37}$ in serum free DMEM with or without phenol red. MTT

reduction was measured after overnight incubation. Figure 10a shows cell viability compared to cells incubated in the absence of hIAPP, in medium with or without phenol red, respectively. Values are mean±SD (n=4),* p=0.03, ** p<0.005. Figure 10b-h are micrographs showing SEM analysis of β cells grown on microscope coverslips under the same conditions. Figures 10b-d show cells following the addition of hIAPP alone. These cells display membrane blebbing and collapse. Figures 10e-f show cells treated with hIAPP in the presence of 40μM phenol red in the growth medium. Figures 10g-h show control cells not treated with hIAPP.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of aromatic compounds which can be used in the treatment of amyloid-associated diseases, such as, for example, diabetes.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Numerous therapeutic approaches for prevention of amyloid fibril formation or disaggreagtion of amyloid material have been described in the prior art. However, current therapeutic approaches are limited by cytotoxicity, non-specificity and delivery barriers.

The present inventors have previously shown that aromatic interactions play a key role in amyloid fibril formation by serving as structural and functional elements that direct molecular recognition and self-assembly [Azriel and Gazit (2001) *J. Biol. Chem* 276: 34156-34161; Gazit (2002) *FASEB J.* 16:77-83]. Consequently, aromatic peptides were shown to inhibit amyloid-fibril formation.

While reducing the present invention to practice, the present inventors uncovered that amyloid formation can be strongly inhibited by aromatic compounds, such as phenol red (PR), suggesting use of these compounds in the treatment of amyloid-associated diseases.

As is illustrated in the Examples section which follows, aromatic compounds of the present invention such as phenol red and derivatives thereof (e.g., diacetoxy phenol red and dimethoxy phenol red), green tea polyphenols, and pyrocatechol violet efficiently inhibited aggregation of an amyloid peptide ($hIAPP_{22-29}$) as determined by a number of biochemical and ultra-structural morphology analyses. In line with this, a remarkable inhibition in $hIAPP_{1-37}$ fibril cytotoxicity towards pancreatic β cells was evident in the presence of phenol red (see Examples 8-9 of the Examples section which follows), rendering the aromatic compounds of the present invention promising tools for treating amyloid associated diseases, such as Type II diabetes mellitus.

Thus, the present invention provides a method of treating an amyloid-associated disease in a subject.

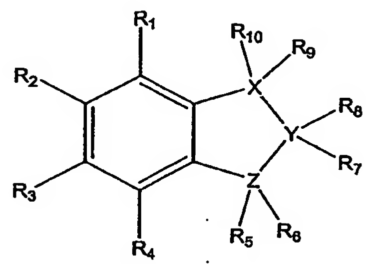
Preferred individual subjects according to the present invention are mammals such as canines, felines, ovines, porcines, equines, and bovines. Preferably the individual subjects according to the present invention are humans.

The term "treating" refers to reducing or preventing amyloid plaque formation, or substantially decreasing plaque occurrence in an affected tissue. The phrase "amyloid plaque" refers to fibrillar amyloid as well as aggregated but not fibrillar amyloid, hereinafter "protofibrillar amyloid", which may be pathogenic as well [see Anaguiano et al. (2002) Biochemistry 41:11338-43].

Amyloid-associated diseases treated according to the present invention include, but are not limited to, type II diabetes mellitus, Alzheimer's disease (AD), early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, Parkinson's disease, SAA amyloidosis, hereditary Icelandic syndrome, multiple myeloma, medullary carcinoma, aortic medical carcinoma, Insulin injection amyloidosis, prion-systematic amyloidosis, choronic inflammation amyloidosis, Huntington's disease, senile systemic amyloidosis, pituitary gland amyloidosis, Hereditary renal amyloidosis, familial British dementia, Finnish hereditary amyloidosis, familial non-neuropathic amyloidosis [Gazit (2002) Curr. Med. Chem. 9:1667-1675] and prion diseases including scrapie of sheep and goats and bovine spongiform encephalopathy (BSE) of cattle [Wilesmith and Wells (1991) Curr Top Microbiol Immunol 172: 21-38] and human prion diseases including (i) kuru, (ii) Creutzfeldt-Jakob Disease (CJD), (iii) Gerstmann-Streussler-Sheinker

Disease (GSS), and (iv) fatal familial insomnia (FFI) [Gajdusek (1977) Science 197: 943-960; Medori, Tritschler et al. (1992) N Engl J Med 326: 444-449].

The method, according to the present invention, is effected by administering to a subject in need thereof, a therapeutically effective amount of a compound having the general Formula I:



Formula I

a pharmaceutically acceptable salt thereof or a prodrug thereof,

wherein:

X, Y and Z are each independently selected from the group consisting of carbon, oxygen, sulfur, $CR_{11}R_{12}$ or $R_{13}R_{14}C-CR_{15}R_{16}$, provided that at least one of X, Y and Z is oxygen or sulfur;

R_1-R_{16} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1-R_4 and/or at least two of R_5-R_{16} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

whereas:

at least one of R_1-R_4 is selected from the group consisting of hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, O-carboxy and O-thiocarboxy; and/or

at least one of R_5-R_{16} comprises phenol, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl hydroxyphenol, and dihydroxyphenol,

The compounds according to the present invention therefore include at least one phenol moiety (preferably at least two phenol moieties). As is further defined hereinbelow, each of the phenol moieties can be either unsubstituted or substituted, preferably by one or more hydroxy groups, thus being hydroxyphenol or dihydroxyphenol. Each of the phenol moieties can be present within the compounds of the present invention either *per se*, namely as a hydroxyphenyl moiety, or as an alkoxylated or carboxylated phenol moiety, namely, as an alkoxyphenyl or carboxyphenyl moiety, as is delineated hereinunder.

As used herein, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms. Whenever a numerical range; *e.g.*, "1-20", is stated herein, it implies that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. More preferably, the alkyl is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, unless otherwise indicated, the alkyl is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined hereinbelow.

A "cycloalkyl" group refers to an all-carbon monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of carbon atoms) group wherein one of more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene, and adamantane. A cycloalkyl group may be substituted or unsubstituted. When

substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined hereinbelow.

A "hydroxy" group refers to an -OH group.

An "alkenyl" group refers to an alkyl group, as defined hereinabove, which consists of at least two carbon atoms and at least one carbon-carbon double bond.

An "alkynyl" group refers to an alkyl group, as defined hereinabove, which consists of at least two carbon atoms and at least one carbon-carbon triple bond.

An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (*i.e.*, rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

A preferred example of a substituted aryl, according to the present invention is phenol.

As used herein, the term "phenol" refers to a phenyl substituted by an hydroxy group. The phenol group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy,

thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

A preferred example of a substituted phenol, according to the present invention, is hydroxyphenol.

As used herein, the term "hydroxyphenol", which also encompasses the term "dihydroxyphenol" refers to a phenol, as defined hereinabove, which is further substituted by one or more additional hydroxy groups. The additional hydroxy groups can be at the *para*, *ortho* and/or *meta* positions with respect to the hydroxy group of the phenol. The hydroxyphenol may be additionally substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

Another preferred examples of a substituted aryl, according to the present invention, include alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl and thioaryloxyphenyl.

As used herein, the term "alkoxyphenyl" refers to a phenyl substituted by an alkoxy group, as defined herein. A representative example of an alkoxy group is methoxy.

The term "thioalkoxyphenyl" refers to a phenyl substituted by a thioalkoxy group, as defined herein.

The term "aryloxyphenyl" refers to a phenyl substituted by an aryloxy group, as defined herein.

The term "thioaryloxyphenyl" refers to a phenyl substituted by a thioaryloxy group, as defined herein.

Each of the alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl and thioaryloxyphenyl groups may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

Preferred substituents of the alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl and thioaryloxyphenyl groups include alkoxy, thioalkoxy, aryloxy and/or thioaryloxy groups, such that examples of preferred substituted alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl and thioaryloxyphenyl include dialkoxyphenyl, dithioalkoxyphenyl, diaryloxyphenyl and dithioaryloxyphenyl, and any other combination.

As used herein, the term "dialkoxyphenyl", refers to an alkoxyphenyl, as defined hereinabove, which is further substituted by one or more additional alkoxy groups. The additional alkoxy groups can be at the *para*, *ortho* and/or *meta* positions with respect to the alkoxy group of the alkoxyphenyl.

The term "dithioalkoxyphenyl", refers to a thioalkoxyphenyl, as defined hereinabove, which is further substituted by one or more additional thioalkoxy groups. The additional thioalkoxy groups can be at the *para*, *ortho* and/or *meta* positions with respect to the thioalkoxy group of the thioalkoxyphenyl.

The term "diaryloxyphenyl", refers to an aryloxyphenyl, as defined hereinabove, which is further substituted by one or more additional aryloxy groups. The additional aryloxy groups can be at the *para*, *ortho* and/or *meta* positions with respect to the aryloxy group of the aryloxyphenyl.

The term "dithioaryloxyphenyl", refers to a thioaryloxyphenyl, as defined hereinabove, which is further substituted by one or more additional thioaryloxy groups. The additional thioaryloxy groups can be at the *para*, *ortho* and/or *meta* positions with respect to the thioaryloxy group of the thioaryloxyphenyl.

Each of the dialkoxyphenyl, dithioalkoxyphenyl, diaryloxyphenyl and dithioaryloxyphenyl may be additionally substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

Another preferred examples of a substituted aryl, according to the present invention, include carboxyphenyl and thiocarboxyphenyl.

As used herein, the term "carboxyphenyl" refers to a phenyl substituted by an O-carboxy group, as defined herein. A representative example of an O-carboxy group is O-acetoxy.

The term "thiocarboxyphenyl" refers to a phenyl substituted by a thiocarboxy group, as defined herein.

The carboxyphenyl and the thiocarboxyphenyl may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

Preferred substituents include additional O-carboxy or thiocarboxy groups, such that examples of preferred substituted carboxyphenyl and thiocarboxyphenyl include dicarboxyphenyl and dithiocarboxyphenyl.

As used herein, the term "dicarboxyphenyl", refers to a carboxyphenyl, e.g., acetoxyphenyl, as defined hereinabove, which is further substituted by one or more

additional carboxy groups. The additional carboxy groups can be at the *para*, *ortho* and/or *meta* positions with respect to the carboxy group of the carboxyphenyl.

The term "dithiocarboxyphenyl", refers to a thiocarboxyphenyl, as defined hereinabove, which is further substituted by one or more additional thiocarboxy groups. The additional thiocarboxy groups can be at the *para*, *ortho* and/or *meta* positions with respect to the thiocarboxy group of the thiocarboxyphenyl.

Each of the dicarboxyphenyl and dithiocarboxyphenyl may be additionally substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

A "heteroaryl" group refers to a monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms, such as, for example, nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups include pyrrole, furane, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline and purine. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms such as nitrogen, oxygen and sulfur. The rings may also

have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic may be substituted or unsubstituted. When substituted, the substituted group can be, for example, lone pair electrons, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein. Representative examples are piperidine, piperazine, tetrahydrofurane, tetrahydropyrane, morpholino and the like.

A "halo" group refers to fluorine, chlorine, bromine or iodine.

An "alkoxy" group refers to both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A "thiohydroxy" group refers to an -SH group.

A "thioalkoxy" group refers to both an -S-alkyl group, and an -S-cycloalkyl group, as defined herein.

An "thioaryloxy" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

An "oxo" group refers to an =O group.

A "carbonyl" group refers to a -C(=O)-R' group, where R' is hydrogen, alkyl, alkenyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) or heteroalicyclic (bonded through a ring carbon) as defined herein.

A "thiocarbonyl" group refers to a -C(=S)-R' group, where R' is as defined herein for R'.

A "C-carboxy" group refers to a -C(=O)-O-R' groups, where R' is as defined herein.

An "O-carboxy" group refers to a R"C(=O)-O- group, where R" is as defined herein.

A "thiocarboxy" group refers to a R"C(=O)-S- group, where R" is as defined herein.

A "sulfinyl" group refers to an -S(=O)-R" group, where R" is as defined herein.

A "sulfonyl" group refers to an $-S(=O)_2-R"$ group, where R" is as defined herein.

A "trihalomethyl" group refers to a -CX group wherein X is a halo group as defined herein.

A "trihalomethanesulfonyl" group refers to a $X_3CS(=O)_2-$ group wherein X is a halo group as defined herein.

A "S-sulfonamido" group refers to a $-S(=O)_2-NR'R''$ group, with R' and R'' as defined herein.

A "N-sulfonamido" group refers to n $R'S(=O)_2-NR''$ group, where R' and R'' are as defined herein.

A "trihalomethanesulfonamido" group refers to an $X_3CS(=O)_2NR'-$ group, where R' and X are as defined herein.

An "O-carbamyl" group refers to an -OC(=O)-NR'R'' group, where R' and R'' are as defined herein.

An "N-carbamyl" group refers to an R''OC(=O)-NR'- group, where R' and R'' are as defined herein.

An "O-thiocarbamyl" group refers to an -OC(=S)-NR'R'' group, where R' and R'' are as defined herein.

An "N-thiocarbamyl" group refers to an R''OC(=S)NR'- group, where R' and R'' are as defined herein.

An "amino" group refers to an –NR'R'' group where R' and R'' are as defined herein.

A "C-amido" group refers to a -C(=O)-NR'R'' group, where R' and R'' are as defined herein.

An "N-amido" group refers to an R'C(=O)-NR'' group, where R' and R'' are as defined herein.

An "urea" group refers to an –NR'C(=O)-NR''R''' group, where R' and R'' are as defined herein and R''' is defined as either R' or R''.

A "guanidino" group refers to an –R'NC(=N)-NR''R''' group, where R', R'' and R''' are as defined herein.

A "guanyl" group refers to an R'R''NC(=N)- group, where R' and R'' are as defined herein.

A "nitro" group refers to an $-NO_2$ group.

A "cyano" group refers to a -C≡N group.

An "azo" group refers to a -N=N group.

The term "phosphonyl" describes a -O-P(=O)(OR')(OR'') group, with R' and R'' as defined hereinabove.

The term "phosphinyl" describes a -PR'R'' group, with R' and R'' as defined hereinabove.

Preferred compounds according to the present invention therefore include, for example, phenol red and analogs thereof, such that in the Formula above X is carbon; Y is oxygen; Z is carbon or sulfur; and at least one of R_5 and R_6 is oxo, as this term is defined hereinabove. Such compounds include a heteroalicyclic ring, fused with phenyl, and further substituted by one or more phenol or phenyl groups, such that at least one of R_5-R_{10} is phenol or hydroxyphenol, as defined hereinabove. Such compounds in which at least one, and preferably two, of R_5-R_{10} are hydroxyphenol include, for example, pyrocatechol violet and analogs thereof.

Compounds in this category, in which Z is sulfur, are typically phenol red analogs, whereas compounds in which Z is carbon are typically phenolphthaleine analogs. The chemical structures of phenol red, pyrocatechol violet and phenolphtalaine are depicted in Figure 7.

Even more preferred compounds according to the present invention, include compounds having the Formula above, in which X is carbon; Y is $R_{13}R_{14}C$-$CR_{15}R_{16}$; and Z is oxygen. Such compounds therefore include a tetrahydropyrane ring fused to phenyl.

Preferred examples of compounds in this category include analogs and derivatives of catechins such as, for example, analogs and derivatives of epicatechin, epigallocatechin, epigallocatechin gallate and the like, all include two hydroxy group at the R_1 and R_3 positions and a hydroxyphenol or dihydroxyphenol group, directly or indirectly attached to the tetrahydropyrane ring, at one or more of the R_{13}-R_{16} positions in the Formula above.

As is shown in the Examples section that follows, catechin gallates, which include an additional phenol moiety, were found to be highly potent inhibitors, thus indicating an important role of the number of the phenyl moieties in the compound.

Additional preferred examples of these compounds include an oxidized tetrahydropyrane ring fused to a phenyl, such that R_9 is oxo; and R_{10} is absent.

Further additional preferred compounds in this category include tocopherol and analogs thereof, which include one or more alkyl groups at the R_{13}-R_{16} positions, whereby the alkyl groups can include lower alkyls (e.g., methyl) and/or alkyls having more than 8 carbon atoms.

Further according to the present invention, each of the compounds described above can further be in a dimeric form. Such a dimeric form includes two moieties having the Formula above, attached therebetween via R_1-R_{16}, directly or indirectly.

Examples of compounds which can be used in accordance with the present invention therefore include, but are not limited to, phenol red, pyrocatechol violet, phenolphthaleine, catechin, epigallocatechin gallate, epicatechin gallate, epicatechin, epigallocatechin, eriodictyol, quercetin, procyanidin, hydroxyphenyl, tocopherol, bromophenol red, analogs thereof, derivatives thereof and any combination thereof.

The presently most preferred compounds according to the present invention are phenol red, pyrocatechol violet and compounds of the catechin gallate family (for further details see the Examples section which follows).

However, additional preferred compounds which can be used in accordance with the present invention include the mono-, di-, tri- and tetra- alkoxy (e.g., methoxy) or carboxy (e.g., acetoxy) derivatives of the compounds listed above. Such derivatives are meant to include compounds in which one or more of the hydroxy groups in the phenol or hydroxyphenol moieties are derivatized by, e.g., an alkyl or acyl group, resulting in an alkoxyphenyl moiety, a dialkoxyphenyl moiety, a carboxyphenyl moiety or a di-carboxyphenyl moiety.

Such a derivatization of the hydroxy groups, which results in the replacement of one or more of the phenol moieties by an alkoxyphenyl moiety, a dialkoxyphenyl moiety, a carboxyphenyl moiety or a di-carboxyphenyl moiety, as well as analogs thereof (e.g., aryloxyphenyl, thioalkoxyphenyl, and the like, as is detailed hereinabove) is highly advantageous since it reduces the hydrophilic nature of the compounds and thus enhances their absorption in the intestines.

As is well known in the art, hydrophilic compounds are typically characterized by relatively low absorption due to poor permeability across human intestinal epithelial. Due to these low absorption parameters, treatment with hydrophilic compounds requires the administration of high doses, when administered orally. Hence, reducing the hydrophilic nature of the compounds described above provides for enhanced absorption thereof, particularly in the intestines, and enables an effective oral administration thereof. The effect of reducing the hydrophilic nature of compounds on their absorption was clearly shown in several models, including the Caco-2 cells and parallel artificial membrane permeation assay (PAMPA). These studies demonstrated that increased hydrophobiciy significantly improves the permeability of small organic compounds [Ano (2004) Bioorg Med Chem. 12:257-264; Ano (2004) 12: 249-255].

Representative examples of such derivatives include, but are not limited to, methoxy phenol red and acetoxy phenol red, in which one phenol moiety in phenol red is replaced by a methoxyphenyl or an acetoxyphenyl moiety, respectively, and dimethoxy phenol red (also referred to herein as PF4) and diacetoxy phenol red (also referred to herein as PF3), in which the two phenol moieties in phenol red are replaced by two methoxyphenyl or acetoxyphenyl moieties, respectively. The chemical structures of PF3 and PF4 are presented in Figure 7.

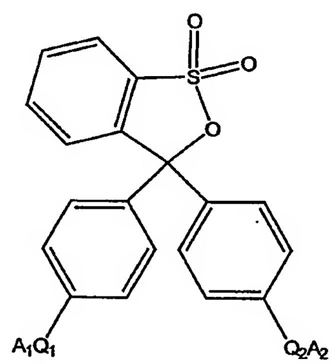
Of a particular importance are the mono derivatives of phenol red, namely, methoxy phenol red and acetoxy phenol red and analogs thereof. These mono derivatives simultaneously provide for (i) enhanced inhibition activity due to the presence of hydroxy groups; (ii) enhanced oral bioavailability due a partial hydrophilic nature thereof; and (iii) enhanced absorption due to a partial hydrophobic nature tehreof, as is detailed hereinabove.

The effect of the hydroxy groups on the inhibition activity of the compounds is demonstrated in the Examples section that follows (see, for example, Example 5). The effect of the hydrophilic nature of compounds on oral bioavailability is well known in the art, and is demonstrated, for example, in Hite et al. (2003) Part 1. Oral Delivery of Poorly Soluble Drugs. PMPS Summer pp:38-40.

Hence, the phenol red mono derivatives of the present invention, by combining enhanced inhibition activity, enhanced oral bioavailability and enhanced absorption, are highly advantageous.

Although some phenol red derivatives, in which the two hydroxy groups are derivatized by alkyl or acyl groups are known, these compounds have never been used for inhibiting amyloid fibril formation. Furthermore, the selective synthesis of the mono derivatives of phenol red mentioned above has never been practiced hitherto.

Hence, according to another aspect of the present invention, there are provided novel phenol red derivatives having the general formula II as follows:



Formula II

wherein:

Q_1 and Q_2 are each independently selected from the group consisting of oxygen and sulfur; and

A_1 and A_2 are each independently selected from the group consisting of hydrogen, alkyl, aryl, cycloalkyl and carbonyl,

whereby when Q_1 and Q_2 are each oxygen, one of A_1 and A_2 is hydrogen and the other is selected from the group consisting of alkyl, cycloalkyl and aryl.

These phenol red derivatives therefore include, for example, methoxy phenol red, in which Q_1 and Q_2 are each oxygen, one of A_1 and A_2 is hydrogen and the other is alkyl, preferably methyl, and acetoxy phenol red, in which Q_1 and Q_2 are each oxygen, one of A_1 and A_2 is hydrogen and the other is carbonyl, preferably acetyl (a $C(=O)CH_3$ group).

The compounds described above can be administered or otherwise utilized in this and other aspects of the present invention, either as is or as a pharmaceutically acceptable salt or a prodrug thereof.

The term "prodrug" refers to an agent, which is converted into the active compound (the active parent drug) *in vivo*. Prodrugs are typically useful for

facilitating the administration of the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility as compared with the parent drug in pharmaceutical compositions. Prodrugs are also often used to achieve a sustained release of the active compound *in vivo*. An example, without limitation, of a prodrug would be a compound of the present invention, having one or more phenol moieties, which is administered as an ester (the "prodrug"). Such a prodrug is hydrolysed *in vivo*, to thereby provide the free compound (the parent drug). The selected ester may affect both the solubility characteristics and the hydrolysis rate of the prodrug.

The phrase "pharmaceutically acceptable salt" refers to a charged species of the parent compound and its counter ion, which is typically used to modify the solubility characteristics of the parent compound and/or to reduce any significant irritation to an organism by the parent compound, while not abrogating the biological activity and properties of the administered compound. An example, without limitation, of a pharmaceutically acceptable salt would be a phenolic anion and a cation such as, but not limited to, ammonium, sodium, potassium and the like.

The compounds described above can further be utilized or administered as hydrates, namely, as a complex of variable stoichiometry (e.g., di-, tri-, tetra-, penta-, hexa-, and so on) formed between the compound and water molecule(s).

Preferably, the compound of the present invention is administered at a concentration not exceeding 4mg/Kg x hr.

The compounds of the present invention can be provided to an individual *per se*, or as part of a pharmaceutical composition where it is mixed with a pharmaceutically acceptable carrier.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to the subject treated.

Herein the term "active ingredient" refers to the compound, which is accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a

carrier or a diluent that does not cause significant irritation to the subject and does not abrogate the biological activity and properties of the administered compound. Preferred carriers of the pharmaceutical composition of the present invention include, but are not limited to, polyethylene glycol (PEG), a biocompatible polymer with a wide range of solubility in both organic and aqueous media (Mutter et al. (1979).

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, inrtaperitoneal, intranasal, or intraocular injections.

Alternately, one may administer a preparation in a local rather than systemic manner, for example, via injection of the preparation directly into a specific region of a patient's body.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal

administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The preparations described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The preparation of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an

amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro assays. For example, a dose can be formulated in animal models and such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. [See e.g., Fingl, et al., (1975) "The Pharmacological Basis of Therapeutics", Ch. 1 p.1].

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions including the preparation of the present invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed

by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert.

It will be appreciated that treatment of amyloid-associated diseases according to the present invention may be combined with other treatment methods known in the art (i.e., combination therapy). Thus, compounds of the present invention may be co-administered (simultaneously or separately) with additional anti-amyloid drugs. Examples of such anti-amyloid drugs include, but are not limited to, amyloid-destabilizing antibodies, amyloid-destabilizing peptides and anti-amyloid small molecules (further details on such drugs are provided in the preceding Background section).

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory

Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

MATERIALS AND METHODS

Peptide synthesis:

Peptide synthesis using solid-phase methods was performed by Peptron, Inc. (Taejeon, Korea) for $hIAPP_{22-29}$ (SEQ ID NO: 1) and for $hIAPP_{20-29}$ (SEQ ID NO: 2), and by Calbiochem (CalBiochem CA, USA) for $hIAPP_{1-37}$ (SEQ ID NO: 3). The identity of the peptides was confirmed by ion spray mass-spectrometry and the purity of the peptides was confirmed by reverse phase high-pressure liquid chromatography (RP-HPLC). The stock solution for $hIAPP_{22-29}$ and $hIAPP_{20-29}$ were prepared by dissolving lyophilized form of the peptides in Me_2SO (DMSO) at a concentration of

50 mM. The stock solution for $hIAPP_{1-37}$ was prepared by dissolving lyophilized form of the peptide in 3,3,3,3',3',3'-hexafluoro-2-propanol (HFIP) at a concentration of 400 μM. To avoid any pre-aggregation the stock solution was sonicated for 2 min before each experiment.

Materials – Phenol red was purchased from Sigma (Rehovot, Israel). Pyrocatechol violet was purchased from Riedel-de Haën (Seelze, Germany). Diacetoxy phenol red (PF3) and dimethoxy phenol red (PF4) were synthesized by TAMI institute for Research & Development Ltd. (Haifa, Israel), Epigallocatechin gallate, Gallocatechin gallate, Gallocatechin, Epigallocatechin, Catechin and Congo red were purchased from SIGMA (Rehovot, Israel), Epicatechin gallate and Epicatechin were purchased from ICN Biomedicals.

Kinetic aggregation assay:

Stock solutions of $hIAPP_{22-29}$ and $hIAPP_{20-29}$ peptide were prepared by dilution of lyophilized peptides into 10 mM Tris-HCl (pH 7.2) buffer to a final concentration of 1 mM peptide and 4% DMSO. Turbidity data were collected at 405 or 560 nm at room temperature. A buffer solution containing the same amount of DMSO as the tested samples was used as blank. Turbidity was measured using disposable UVette cuvettes (Eppendorf, Germany) and a Scinco S-3100 spectrophotometer.

Thioflavin T fluorescence assay:

Fibrillization of $hIAPP_{1-37}$ was monitored by Thioflavin T dye binding assay. $hIAPP_{1-37}$ stock solution was diluted to a final concentration of 4 μM in 10 mM sodium acetate buffer (pH 6.5) with or without inhibitor, and a final HFIP concentration of 1% (vol). Immediately after dilution, sample was centrifuged for 20 minutes in 20,000g at 4 °C and the supernatant fraction was used for the fluorescence measurements. In every measurement ThT was added to a final concentration of 3 μM and measurements were carried out using Perkin Elmer (excitation 450 nm, 2.5 nm slit; emission 480 nm 10 nm slit). For the phenol red concentration dependent inhibition experiments, samples were diluted 10 fold such that maximal phenol red concentration did not exceed 4μM, and measured using Jobin Yvon Horiba Fluoromax 3 fluorimeter (excitation 450 nm, 2.5 nm slit; emission 482 nm, 5 nm slit). Background was subtracted from all samples.

Circular Dichroism Spectroscopy:

To follow secondary structure formation, $hIAPP_{1-37}$ (4 μM) was prepared as mentioned above, with or without inhibitor (40 μM). Spectra were recorded at 25 °C between 200-250 nm, with 1 nm intervals and 4 sec averaging time, using an AVIV 202 CD spectrometer. Final scan values represent subtraction of base line (buffer in case of hIAPP, and buffer with phenol red for the inhibition assay) and smoothing using AVIV CDS version 2.73 software.

Transmission Electron Microscopy:

10 μL samples of $hIAPP_{22-29}$ or $hIAPP_{20-29}$ from the aggregation assay, and $hIAPP_{1-37}$ from the fluorescence assay were placed on 400-mesh copper grids (SPI supplies, West Chester PA) covered by carbon-stabilized Formvar film. After 1 minute, excess fluid was removed, and the grids were negatively stained with 2% uranyl acetate in water for another two minutes. Samples were viewed in a JEOL 1200EX electron microscope operating at 80 kV.

MTT assay – βTC-tet cells [Fleischer, N., Chen C., Surana, M., Leiser, M., Rossetti, L., Pralong, W. and Efrat, S. (1998) Functional analysis of a conditionally transformed pancreatic beta-cell line, *Diabetes 47*, 1419-1425] or PC12 cells (ATCC # CRL-1721) were plated on 24-well plates ($2 \cdot 10^5$/well) or on 96-well plates ($1 \cdot 10^4$/well) respectively, and allowed to adhere for 24 hours. Synthetic $hIAPP_{1-37}$ stock solution was diluted to a final concentration of 4 μM in serum free growth medium [Fleischer (1998) Supra] containing DMEM with or without phenol red. Immediately following dilution, samples were centrifuged at 4 °C for 20 minutes at 20,000g, and the supernatant was bubbled with nitrogen for 30 min to evaporate residual HFIP. Cells were washed twice with PBS and incubated with the supernatant for 24 hrs. MTT (Sigma M-2128) was then added for 3 hrs, followed by addition of lysis buffer and incubation over night. Assay was effected according to Manufacturer's instructions. Samples were read at 570 nm. Cell viability was calculated in comparison to cells incubated in the absence of hIAPP in medium with or without phenol red, respectively.

Scanning electron microscopy – Cells were grown on glass microscope cover slips under the same conditions as for the MTT assay. Immediately following incubation with hIAPP, the cells were fixed with 2% glutaraldehide (v/v) and stored for 24 hrs at 4 °C. The cells were serially dehydrated with increasing concentrations

of ethanol (30%, 50%, 70%, 90%, 95%, 100%) and dried with a critical point drier. Specimen cover slips were coated with colloidal gold and viewed using a JOEL JSM 840A microscope operating at 25 kV.

EXAMPLE 1

Effect of phenol red on the aggregation of the core amyloidogenic fragments of hIAPP

The core amyloidogenic fragments of hIAPP ($hIAPP_{22\text{-}29}$, $hIAPP_{20\text{-}29}$) aggregate in an aqueous solution. Such an aggregation behavior was served as a preliminary assay for amyloid formation.

In order to evaluate the ability of phenol red to inhibit amyloid formation, the aggregation of 1 mM $hIAPP_{22\text{-}29}$ and $hIAPP_{20\text{-}29}$ (SEQ ID NO: 1 and NO: 2, respectively) was examined in the absence or presence of 40 mM phenol red. To verify the specificity of phenol red molecule towards hIAPP core peptides, a very similar molecule – phenolphthaleine, which differs only by the lack of the sulfon group, was used as control (Figure 7).

As shown in Figures 1a-b, $hIAPP_{20\text{-}29}$ aggregation rate was lower than $hIAPP_{22\text{-}29}$, and approximately two hours lag time was evident in all samples. No inhibition effect was evident using phenolphthaleine and its aggregation curve was similar to $hIAPP_{20\text{-}29}$ aggregation which increased dramatically after three hours (Figure 1b). Using phenol red as an inhibitor decreased aggregation showing very minor elevation in aggregation levels after 3 hours of incubation.

As is shown in Figure 1a, $hIAPP_{22\text{-}29}$ aggregated within seconds and its turbidity reached a plateau following 10 minutes of incubation. However, in the presence of phenol red, aggregation of $hIAPP_{22\text{-}29}$ was substantially terminated exhibiting much lower rate and constant levels of turbidity.

EXAMPLE 2

Secondary structure evaluation using Circular Dichroism (CD)

Wild type $hIAPP_{1\text{-}37}$ (SEQ ID NO: 3) undergoes a transition from random coil conformation to β sheet within the fibrillization process. This secondary structure transition was addressed in the presence or absence of phenol red. $hIAPP_{1\text{-}37}$ was dissolved in HFIP and diluted into sodium acetate buffer (pH 6.5) to a final

concentration of 4 μM and 1% HFIP. Changes in the peptide secondary structure were monitored for 96 hours.

As shown in Figure 2a, in the absence of phenol red, $hIAPP_{1-37}$ showed an initial transition to β sheet structure within 6 hours and maximal ellipticity (218 nm, which corresponds to β sheet structures) within 24 hours. These structures remained relatively steady for 96 hours.

Upon the addition of 10 molar ratio of phenol red, transition to β sheet conformation was significantly inhibited (Figure 2b), wherein initial very low levels of 218 nm ellipticity were measured after 46 hours with very slow enhancement after 96 hours.

EXAMPLE 3

Amyloid fibril formation by Thioflavin T fluorescence

Thioflavin T (ThT) is commonly used for detecting the level of amyloid formation. $hIAPP_{1-37}$ solution was prepared as described above. Following incubation in the presence or absence of phenol red and addition of 3 μM ThT to each sample, fluorescence was measured.

As is shown in Figure 3a, in the absence of phenol red, $hIAPP_{1-37}$ displayed a lag phase of approximately 20 hours that was followed by a fast enhancement in fluorescent levels. In the presence of 40 μM phenol red, very low and constant levels of fluorescence were detected for throughout the assay. A dose dependent inhibitory effect of phenol red on aggregation of the hIAPP peptide is shown in Figures 3b-c. Higher phenol red concentrations (higher than 4 fold of phenol red to hIAPP) have shown constant low fluorescence levels following 24 hours (Figure 3b). Even after one week of incubation, the concentration dependent inhibition was similar (Figure 3c), and inhibition level of about 90 % was achieved for phenol red concentration of 20 μM and above. These results substantiate that phenol red is a potent inhibitor of amyloid fibril formation.

EXAMPLE 4

Morphology of hIAPP fibrils with phenol red as determined by TEM

Samples of $hIAPP_{22-29}$ and $hIAPP_{20-29}$ taken from the aggregation assay (described in Example 3) were visualized using transmission electron microscopy. As

is shown in Figure 4a, distinct and well-defined amyloid fibers were observed 3 and 72 hours after initiation of aggregation in both samples of the $hIAPP_{22-29}$ peptide, with a minor increase in fiber density and width following 72 hours. In contrast, in the presence of phenol red, no fibers were visualized following 3 hours while only small amount of fibrils, with different morphology were visualized after 72 hours. Same results were obtained with the $hIAPP_{20-29}$ peptide after 24 hr incubation (Figure 4b) in the presence of phenol red inhibitor. No inhibition was observed using phenolphthaleine under the same conditions and fibrillar structures were evident on the TEM grid.

As is shown in Figure 4c, the same inhibitory tendency was observed when using phenol red as an inhibitor of $hIAPP_{1-37}$ amyloidosis. A significant kinetic inhibition in fiber formation was present in the first stage of fiber formation (approximately 30 hours) where no fibers where present in presence of phenol red.

EXAMPLE 5

Inhibition of IAPP fibril formation with green tea polyphenols

ThT was used to determine the inhibitory effect of green tea polyphenol compounds on $hIAPP_{1-37}$ fibril formation (See Example 3).

Results:

As is shown in Figure 5a, all polyphenol compounds had a long-term inhibitory effect on $hIAPP_{1-37}$ fibril formation as compared to the hIAPP alone. This inhibition resembled the inhibitory effect of congo red. Fluorescence values of IAPP alone increased after 48 hr while an initial increase of $hIAPP_{1-37}$ fluorescence in the presence of inhibitors was detectable only after 72 hr.

A more detailed observation of the inhibitory effect revealed that all poly phenols with the gallate group (i.e., containing an additional phenolic ring) are better inhibitors (Figure 5b). This may suggest that there is an importance to the additional aromatic ring on the inhibitory effect. This result was independently confirmed by two other groups [Lashuel et al *J. Biol. Chem.* 2002 277:42881-90; Kocisco et al *J. Virol.* 2004 77: 10288-10294] describing the inhibitory effect of Apomorphines on β-amyloid and the inhibitory effect of various polyphenols on scrapie –associated prion protein.

EXAMPLE 6

Dose-dependent inhibition of human islet amyloid polypeptide fibril formation by pyrocatechol violet

Experimental procedures

Thioflavin T fluorescence assay: Fibrillization of $hIAPP_{1-37}$ was monitored by Thioflavin T (ThT) dye binding assay, as described above, using an inhibitor concentration of 40 μM or 4 μM.

Results

As is shown in Figure 6, the results obtained in the ThT assay clearly show that pyrocatechol violet effectively inhibit amyloid formation by $hIAPP_{1-37}$. While hIAPP without pyrocatechol violet displayed a quick increase in fluorescence levels, as demonstrated by a peak value already at the first time point (2.6 hours), addition of 4 μM pyrocatechol violet resulted in a significant lower level of fluorescence throughout the entire assay, whereby an even stronger inhibitory effect was detected upon addition of 40 μM pyrocatechol violet.

EXAMPLE 7

Inhibition of IAPP fibril formation by phenol red derivates

To evaluate the ability of the phenol red derivatives described hereinabove PF3 (diacetoxy phenol red, Figure 7) and PF4 (dimethoxy phenol red, Figure 7) to inhibit fibril formation as compared to phenol red, a fluorescence ThT kinetic assay was effected.

Results

As is evident from Figures 8a-b, both PF3 and PF4 (40 μM) molecules have exhibited reduced inhibition effect relatively to the phenol red molecule. PF3 molecule was found not stable and tended to fragmentize within few minutes of solubilization. PF4 molecule relative inhibitory effect was more evident, especially when the sample was not centrifuged (Figure 8b).

EXAMPLE 8

Phenol red elicits survival effect on PC12 cells treated with hIAPP

To study the effect of phenol red on cytotoxicity induced by $hIAPP_{1-37}$ aggregates, the PC12 cell line was used. Cells were grown in a 96 well plate with or

without gradual concentration of phenol red, while adding fresh $hIAPP_{1-37}$ to the growth medium. Following 24 hours incubation, cell viability was calculated by substitution of IAPP samples absorbance with non IAPP controls containing the same phenol red concentrations. As is evident from Figure 9, the MTT cell viability assay showed concentration dependent rescue of the cells by phenol red.

EXAMPLE 9

Phenol red inhibits the cytotoxic effect of amyloid aggregates on pancreatic β cells

The ability of phenol red to modulate cytotoxicity effect of hIAPP amyloid assemblies on pancreatic β cells in culture was addressed. A highly-differentiated murine β-cell line (βTC-tet, Fleischer 1998 SUpra) with a normal insulin secretory response to glucose was used. Cells were grown with or without phenol red, and fresh hIAPP was added to the growth medium. An MTT cell viability assay clearly revealed that the presence of phenol red in the medium protected β cells from the cytotoxic effect of hIAPP assemblies and increased cell viability from 50% to 80% ($P<0.05$) (Figure 10a). Scanning electron microscopy (SEM) analysis of β cells that were grown in the presence of hIAPP showed an extensive membrane blebbing (Figure 10b), as previously reported for hIAPP cytotoxicity [Saafi, E. L., Konarkowska, B., Zhang, S., Kistler, J. and Cooper, G. J. (2001) Ultrastructural evidence that apoptosis is the mechanism by which human amylin evokes death in RINm5F pancreatic islet beta-cells, *Cell. Biol. Int. 25*, 339-350], and a collapse of typical cellular morphology in the vast majority of cells (Figure 10d). On the other hand, practically no significant difference could be observed between untreated cells (Figure 10h) and cells grown in the presence of hIAPP and phenol red (Figure 10f). In both cases, most of the cells maintained normal morphology. No blebbing was visible, and membrane extensions of microspikes and lamellipodia were present. Furthermore, at low magnification SEM examination, normal arrays of β-cells could be observed with the phenol red protected cells and control cells (Figures 10e and 10g, respectively). In marked contrast, only isolated and morphologically altered cells could be observed upon IAPP incubation with no phenol red protection (Figure 10c).

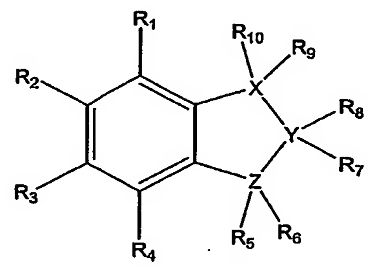
Taken together these results indicate that phenol red inhibit the cytotoxic effect of $hIAPP_{1-37}$ fibrils on cells, particularly pancreatic β cells.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

WHAT IS CLAIMED IS:

1. Use of a compound having the general Formula I:



Formula I

a pharmaceutically acceptable salt thereof or a prodrug thereof,

wherein:,

X, Y and Z are each independently selected from the group consisting of carbon, oxygen, sulfur, $CR_{11}R_{12}$ or $R_{13}R_{14}C$-$CR_{15}R_{16}$, provided that at least one of X, Y and Z is oxygen or sulfur; and

R_1-R_{16} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl,. or absent, or, alternatively, at least two of R_1-R_4 and/or at least two of R_5-R_{16} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

whereas:

at least one of R_1-R_4 is selected from the group consisting of hydroxy, thiohydroxy, alkoxy, thioalkoxy, aryloxy, thioaryloxy, carboxy and thiocarboxy; and/or

at least one of R_5-R_{16} comprises phenol, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, hydroxyphenol, and dihydroxyphenol,

with the proviso that the compound is not any one of catechin, epicatechin, gallocatechin gallate, epigallocatechin gallate, epigallocatechin, and epicatechin gallate,

for the manufacture of a medicament identified for the treatment of amyloid-associated diseases.

2. The use of claim 1, wherein:
X is carbon;
Y is $R_{13}R_{14}C-CR_{15}R_{16}$; and
Z is oxygen.

3. The use of claim 2, wherein:
R_9 is oxo; and
R_{10} is absent.

4. The use of claim 2 or 3, wherein at least one of R_{13}-R_{16} is selected from the group consisting of alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol and dihydroxyphenol.

5. The use of claim 4, wherein each of R_1 and R_3 is hydroxy.

6. The use of claim 2, wherein at least one of R_{13}-R_{16} is alkyl.

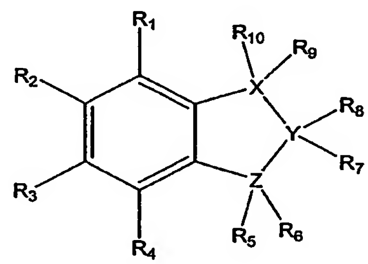
7. The use of claim 1, wherein:
X is carbon;
Y is oxygen;
Z is carbon or sulfur; and
at least one of R_5 and R_6 is oxo.

8. The use of claim 7, wherein at least one of R_9 and R_{10} is selected from the group consisting of alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl,

thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol and dihydroxyphenol.

9. The use of claim 1, wherein said compound is selected from the group consisting of phenol red, dimethoxy phenol red, methoxy phenol red, diacetoxy phenol red, acetoxy phenol red, pyrocatechol violet, phenolphthaleine, catechin, epigallocatechin gallate, epicatechin gallate, epicatechin, epigallocatechin, eriodictyol, quercetin, procyanidin, hydroxyphenyl, tocopherol, and bromophenol red.

10. An article-of-manufacture comprising a packaging material and a pharmaceutical composition identified for treating amyloid-associated diseases being contained within said packaging material, said pharmaceutical composition including, as an active ingredient, a compound having the general Formula I:



Formula I

a pharmaceutically acceptable salt thereof or a prodrug thereof,

wherein:

X, Y and Z are each independently selected from the group consisting of carbon, oxygen, sulfur, $CR_{11}R_{12}$ or $R_{13}R_{14}C\text{-}CR_{15}R_{16}$, provided that at least one of X, Y and Z is oxygen or sulfur; and

R_1-R_{16} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1-R_4 and/or at least two of

R_5-R_{16} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

whereas:

at least one of R_1-R_4 is selected from the group consisting of hydroxy, thiohydroxy, alkoxy, thioalkoxy, aryloxy, thioaryloxy, carboxy and thiocarboxy; and/or

at least one of R_5-R_{16} comprises phenol, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, hydroxyphenol, and dihydroxyphenol,

with the proviso that the compound is not any one of catechin, epicatechin, gallocatechin gallate, epigallocatechin gallate, epigallocatechin, and epicatechin gallate,

and a pharmaceutically acceptable carrier.

11. The article-of-manufacture of claim 10, wherein:

X is carbon;

Y is $R_{13}R_{14}C$-$CR_{15}R_{16}$; and

Z is oxygen.

12. The article-of-manufacture of claim 11, wherein:

R_9 is oxo; and

R_{10} is absent.

13. The article-of-manufacture of claim 11 or 12, wherein at least one of R_{13}-R_{16} is selected from the group consisting of alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol and dihydroxyphenol.

14. The article-of-manufacture of claim 13, wherein each of R_1 and R_3 is hydroxy.

15. The article-of-manufacture of claim 11, wherein at least one of R_{13}-R_{16} is alkyl.

16. The article-of-manufacture of claim 10, wherein:

X is carbon;

Y is oxygen;

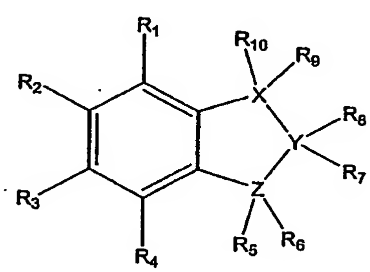
Z is carbon or sulfur; and

at least one of R_5 and R_6 is oxo.

17. The article-of-manufacture of claim 16, wherein at least one of R_9 and R_{10} is selected from the group consisting of alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol and dihydroxyphenol.

18. The article-of-manufacture of claim 10, wherein said compound is selected from the group consisting of phenol red, dimethoxy phenol red, methoxy phenol red, diacetoxy phenol red, acetoxy phenol red, pyrocatechol violet, phenolphthaleine, catechin, epigallocatechin gallate, epicatechin gallate, epicatechin, epigallocatechin, eriodictyol, quercetin, procyanidin, hydroxyphenyl, tocopherol, and bromophenol red.

19. A method of treating an amyloid-associated disease in a subject, the method comprising administering to a subject in need thereof, a therapeutically effective amount of a compound having the general Formula I:



Formula I

a pharmaceutically acceptable salt thereof or a prodrug thereof,

wherein,

X, Y and Z are each independently selected from the group consisting of carbon, oxygen, sulfur, $CR_{11}R_{12}$ or $R_{13}R_{14}C\text{-}CR_{15}R_{16}$, provided that at least one of X, Y and Z is oxygen or sulfur; and

R_1-R_{16} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1-R_4 and/or at least two of R_5-R_{16} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

whereas,

at least one of R_1-R_4 is selected from the group consisting of hydroxy, thiohydroxy, alkoxy, thioalkoxy, aryloxy, thioaryloxy, carboxy and thiocarboxy; and/or

at least one of R_5-R_{16} comprises phenol, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, hydroxyphenol, and dihydroxyphenol,

with the proviso that the compound is not any one of catechin, epicatechin, gallocatechin gallate, epigallocatechin gallate, epigallocatechin, and epicatechin gallate,

thereby treating the amyloid-associated disease in the subject.

20. The method of claim 19, wherein said administering is effected at a concentration of said compound not exceeding 4mg/Kg body weight/hour.

21. The method of claim 19, wherein said administering is effected orally.

22. The method of claim 19, wherein:

X is carbon;

Y is $R_{13}R_{14}C\text{-}CR_{15}R_{16}$; and

Z is oxygen.

23. The method of claim 22, wherein:
R_9 is oxo; and
R_{10} is absent.

24. The method of claim 22 or 23, wherein at least one of R_{13}-R_{16} is selected from the group consisting of alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol and dihydroxyphenol.

25. The method of claim 24, wherein each of R_1 and R_3 is hydroxy.

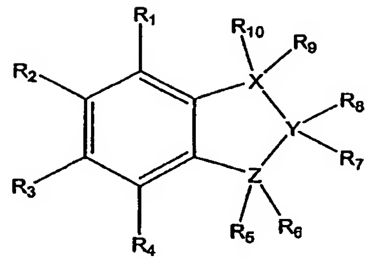
26. The method of claim 22, wherein at least one of R_{13}-R_{16} is alkyl.

27. The method of claim 19, wherein:
X is carbon;
Y is oxygen;
Z is carbon or sulfur; and
at least one of R_5 and R_6 is oxo.

28. The method of claim 27, wherein at least one of R_9 and R_{10} is selected from the group consisting of alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol and dihydroxyphenol.

29. The method of claim 19, wherein said compound is selected from the group consisting of phenol red, dimethoxy phenol red, methoxy phenol red, diacetoxy phenol red, acetoxy phenol red, pyrocatechol violet, phenolphthaleine, catechin, epigallocatechin gallate, epicatechin gallate, epicatechin, epigallocatechin, eriodictyol, quercetin, procyanidin, hydroxyphenyl, tocopherol, and bromophenol red.

30. A pharmaceutical composition, for use in the treatment of amyloid-associated diseases, comprising a therapeutically effective amount of a compound having the general Formula I:



Formula I

a pharmaceutically acceptable salt thereof or a prodrug thereof,

wherein,

X, Y and Z are each independently selected from the group consisting of carbon, oxygen, sulfur, $CR_{11}R_{12}$ or $R_{13}R_{14}C\text{-}CR_{15}R_{16}$, provided that at least one of X, Y and Z is oxygen or sulfur; and

R_1-R_{16} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1-R_4 and/or at least two of R_5-R_{16} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

whereas:

at least one of R_1-R_4 is selected from the group consisting of hydroxy, thiohydroxy, alkoxy, thioalkoxy, aryloxy, thioaryloxy, carboxy and thiocarboxy; and/or

at least one of R_5-R_{16} comprises phenol, alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, hydroxyphenol, and dihydroxyphenol,

with the proviso that the compound is not any one of catechin, epicatechin, gallocatechin gallate, epigallocatechin gallate, epigallocatechin, and epicatechin gallate,

and a pharmaceutically acceptable carrier.

31. The pharmaceutical composition of claim 30, wherein:
X is carbon;
Y is $R_{13}R_{14}C-CR_{15}R_{16}$; and
Z is oxygen.

32. The pharmaceutical composition of claim 31, wherein:
R_9 is oxo; and
R_{10} is absent.

33. The pharmaceutical composition of claim 31 or 32, wherein at least one of R_{13}-R_{16} is selected from the group consisting of alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol and dihydroxyphenol.

34. The pharmaceutical composition of claim 33, wherein each of R_1 and R_3 is hydroxy.

35. The pharmaceutical composition of claim 31, wherein at least one of R_{13}-R_{16} is alkyl.

36. The pharmaceutical composition of claim 30, wherein:
X is carbon;
Y is oxygen;
Z is carbon or sulfur; and
at least one of R_5 and R_6 is oxo.

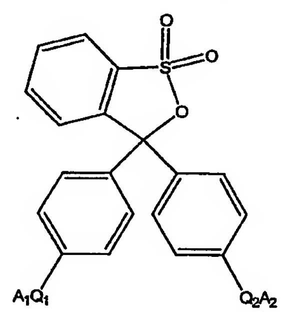
37. The pharmaceutical composition of claim 36, wherein at least one of R_9 and R_{10} is selected from the group consisting of alkoxyphenyl, thioalkoxyphenyl, aryloxyphenyl, thioaryloxyphenyl, carboxyphenyl, thiocarboxyphenyl, phenol, hydroxyphenol and dihydroxyphenol.

38. The pharmaceutical composition of claim 30, wherein said compound is selected from the group consisting of phenol red, dimethoxy phenol red, methoxy phenol red, diacetoxy phenol red, acetoxy phenol red, pyrocatechol violet, phenolphthaleine, catechin, epigallocatechin gallate, epicatechin gallate, epicatechin, epigallocatechin, eriodictyol, quercetin, procyanidin, hydroxyphenyl, tocopherol, and bromophenol red.

39. The pharmaceutical composition of claim 30, further comprising an anti-amyloid drug.

40. The pharmaceutical composition of claim 39, wherein said anti-amyloid drug is selected from the group consisting of an amyloid-destabilizing antibody, an amyloid-destabilizing peptide and an anti-amyloid small molecule.

41. A compound having the general formula II:



Formula II

a pharmaceutically acceptable salt thereof or a prodrug thereof,

wherein:

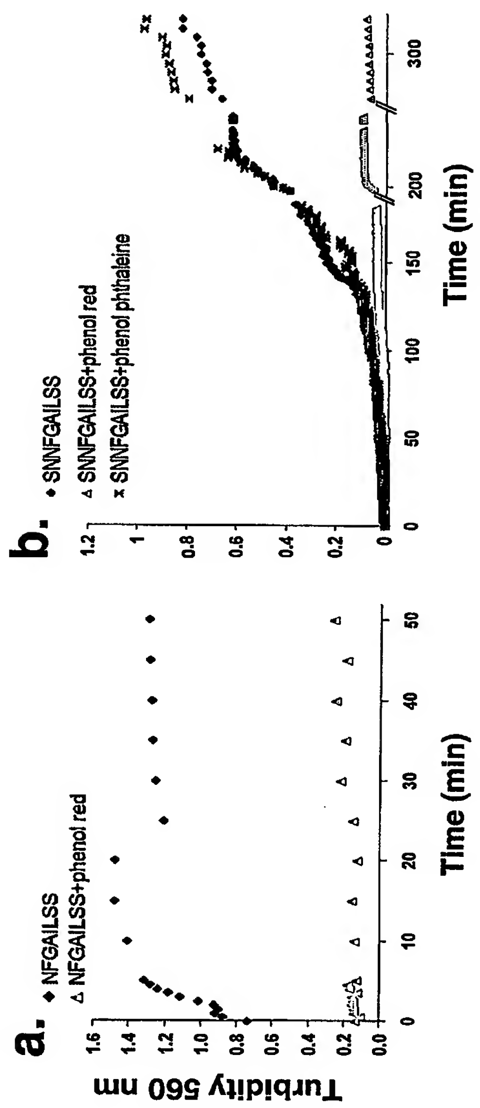
Q_1 and Q_2 are each independently selected from the group consisting of oxygen and sulfur; and

A_1 and A_2 are each independently selected from the group consisting of hydrogen, alkyl, aryl, cycloalkyl and carbonyl,

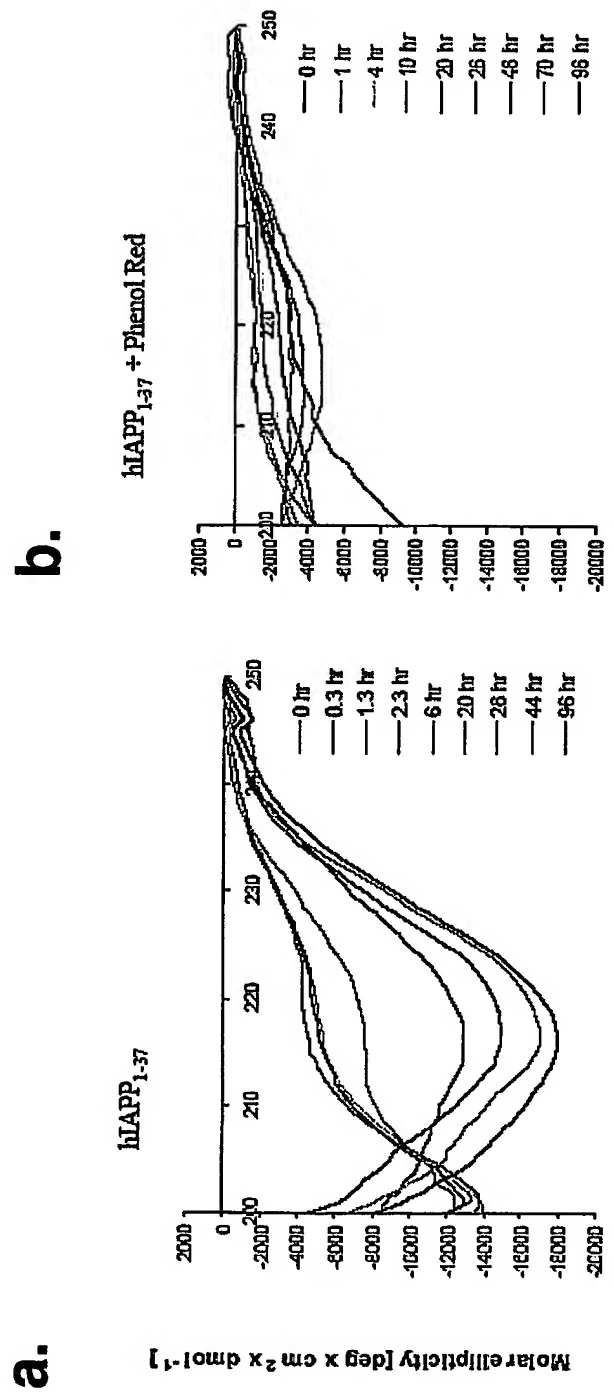
whereas when Q_1 and Q_2 are each oxygen, one of A_1 and A_2 is hydrogen and the other is selected from the group consisting of alkyl, cycloalkyl, aryl and carbonyl.

42. The compound of claim 41, wherein Q_1 and Q_2 are each oxygen, one of A_1 and A_2 is hydrogen and the other is methyl.

43. The compound of claim 41, wherein Q_1 and Q_2 are each oxygen, one of A_1 and A_2 is hydrogen and the other is acetyl.


a.
NFGAILSS
NFGAILSS+phenol red
Turbidity 560 nm
Time (min)
b.
SNNFGAILSS
SNNFGAILSS+phenol red
SNNFGAILSS+phenol phthaleine
Time (min)

Figures 1a-b


a.
hIAPP$_{1-37}$
Molar ellipticity [deg x cm^2 x dmol^{-1}]
0 hr
0.3 hr
1.3 hr
2.3 hr
6 hr
20 hr
28 hr
44 hr
96 hr
b.
hIAPP$_{1-37}$ + Phenol Red
0 hr
1 hr
4 hr
10 hr
20 hr
26 hr
48 hr
70 hr
96 hr

Figures 2a-b

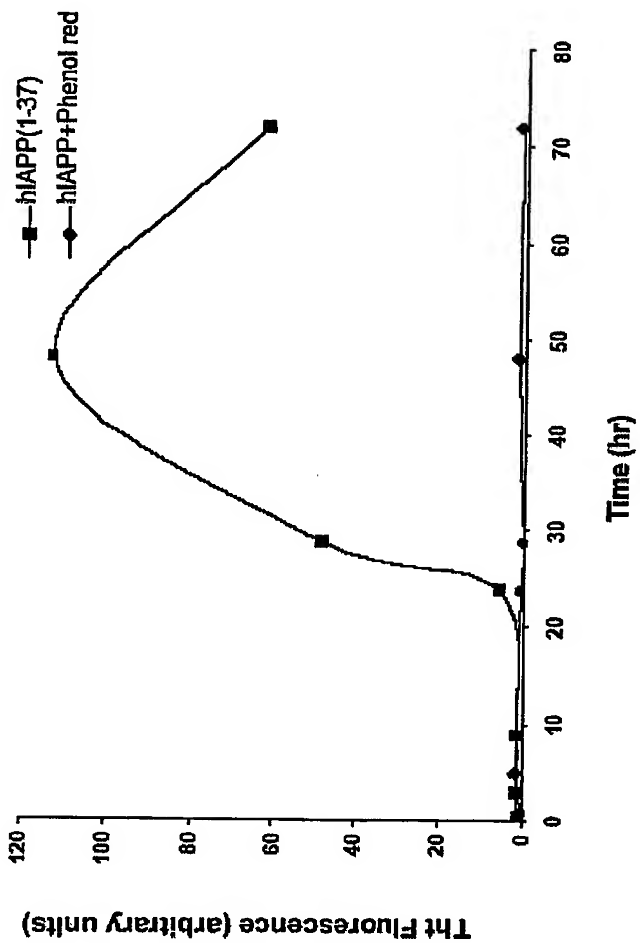

hIAPP(1-37)
hIAPP+Phenol red
ThT Fluorescence (arbitrary units)
Time (hr)
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70
80

Figure 3a

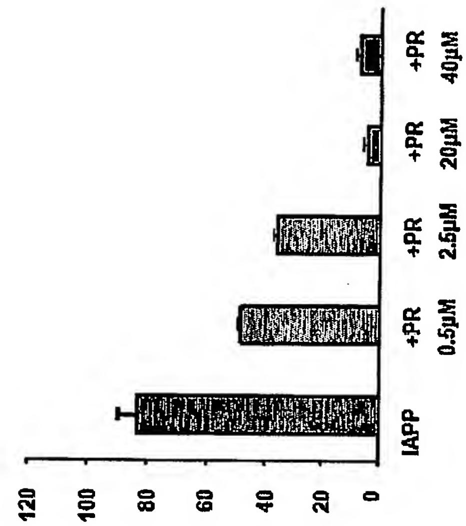


Figure 3c

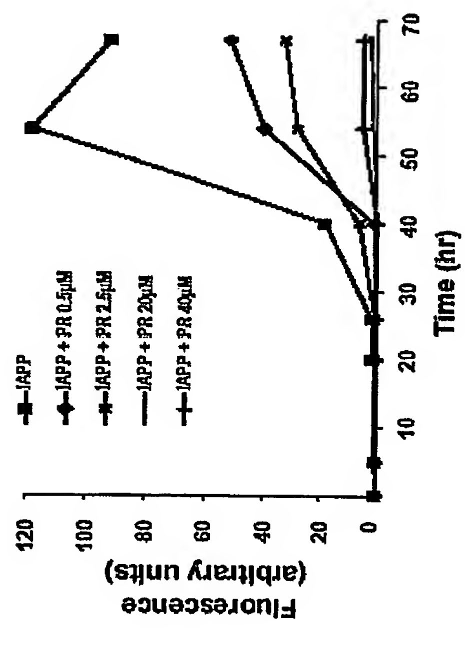


Figure 3b

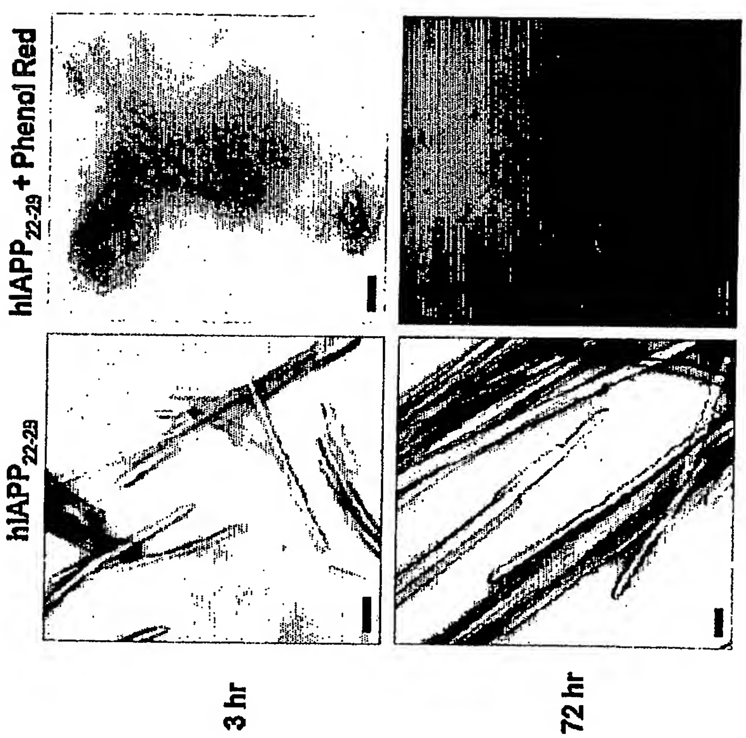


Figure 4a

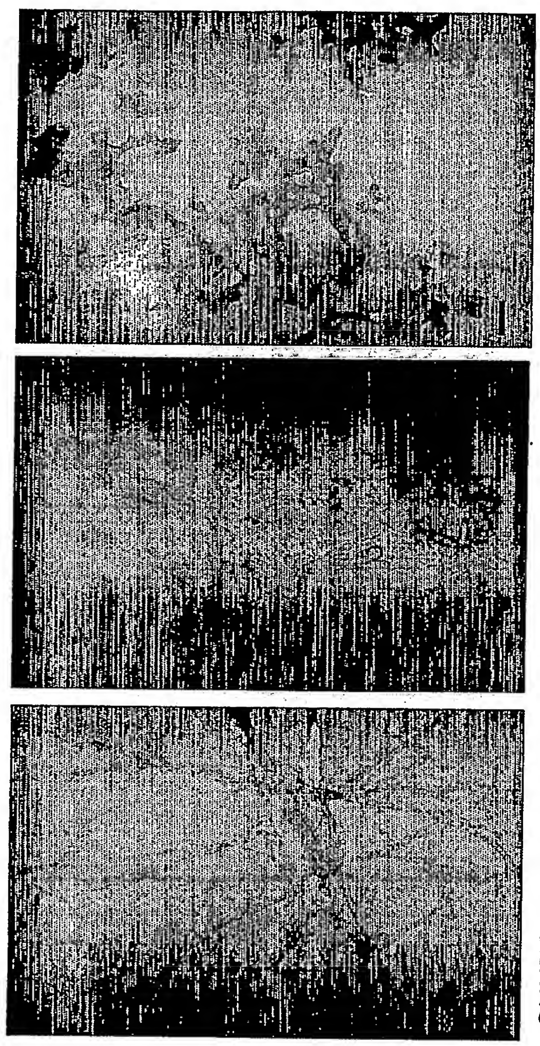

SNNFGAILSS 1mM+
phenol red 10mM
SNNFGAILSS 1mM+
phenolphthaleine 10mM
SNNFGAILSS 1mM

Figure 4b

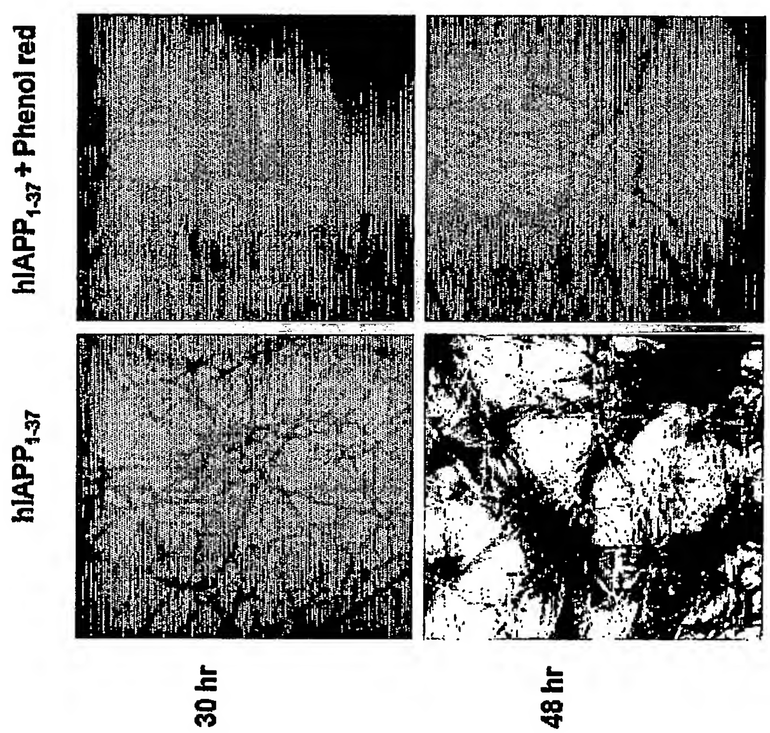


Figure 4c

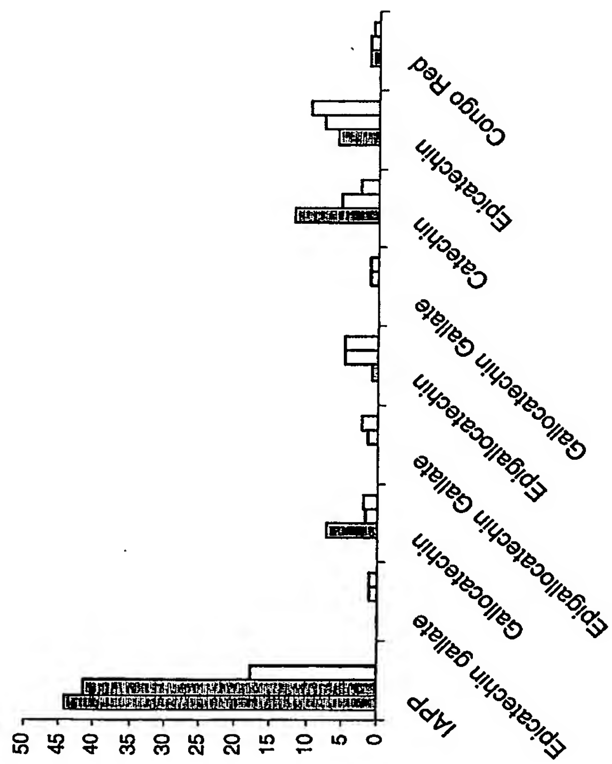

50
45
40
35
30
25
20
15
10
5
0
IAPP
Epicatechin gallate
Gallocatechin
Epigallocatechin Gallate
Epigallocatechin
Gallocatechin Gallate
Catechin
Epicatechin
Congo Red

Figure 5a

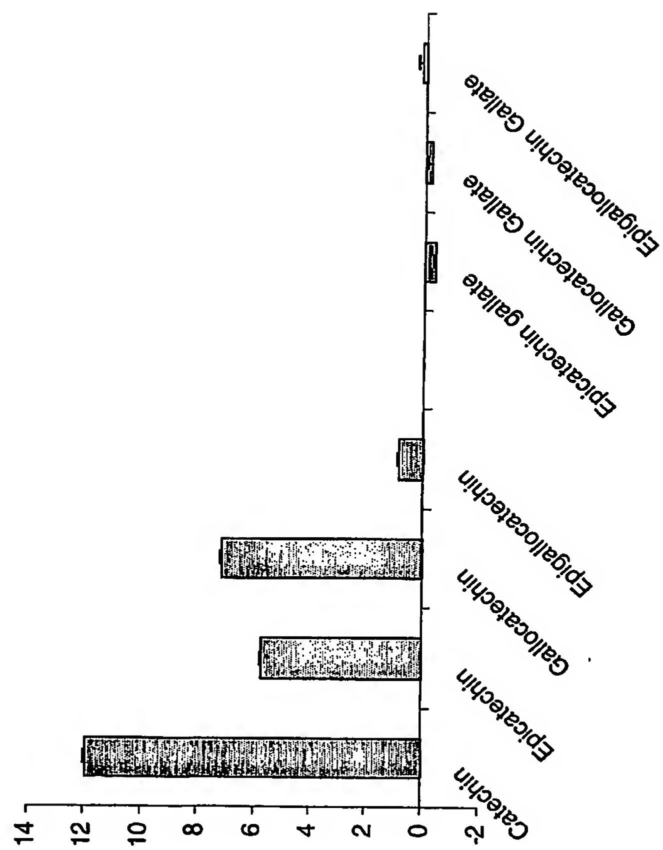

14
12
10
8
6
4
2
0
-2
Catechin
Epicatechin
Gallocatechin
Epigallocatechin
Epicatechin gallate
Gallocatechin Gallate
Epigallocatechin Gallate

Figure 5b

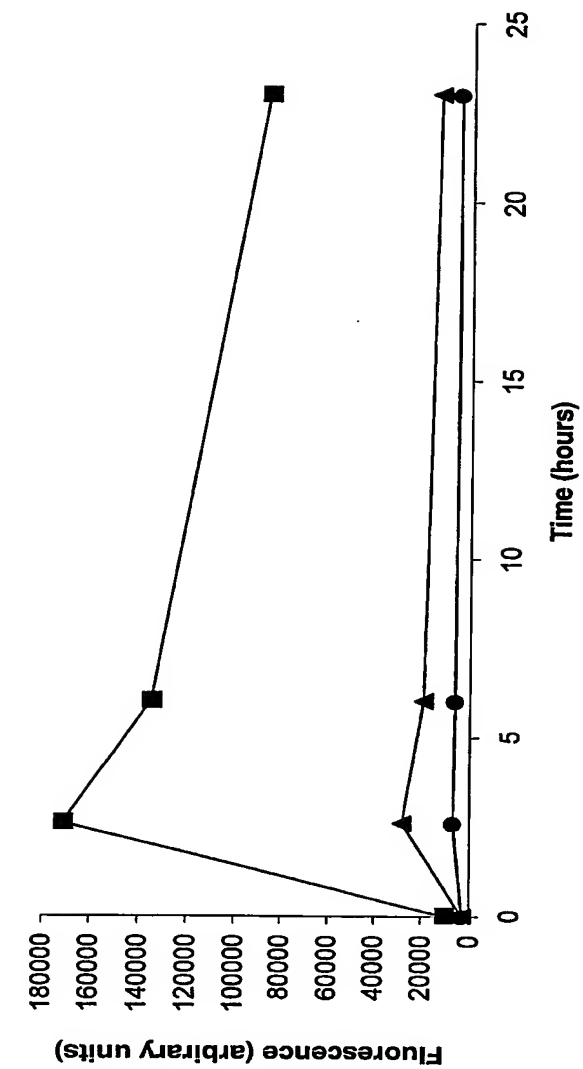
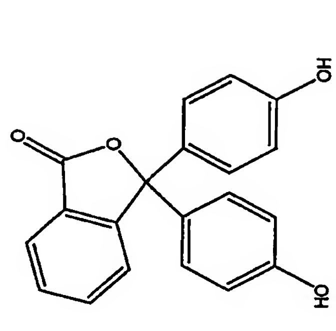
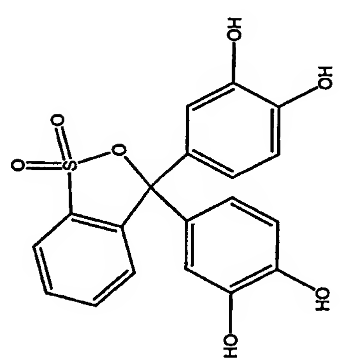

Fluorescence (arbirary units)
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0
5
10
15
20
25
Time (hours)

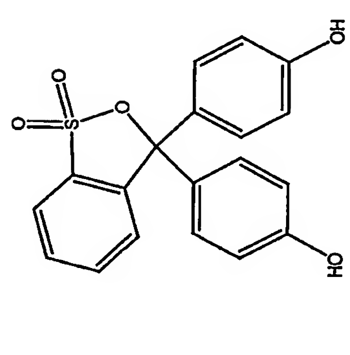
Figure 6



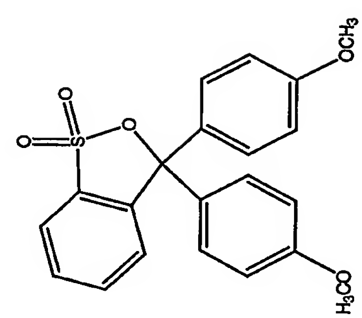
Phenolphthaleine



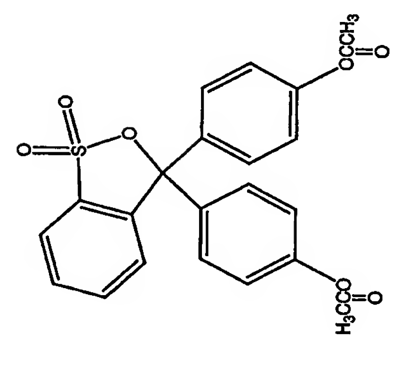
Pyrocatechol



Phenolsulfonphthaleine (phenol red)



Dimethoxy phenol red (PF4)



Diacetoxy phenol red (PF3)

Figure 7

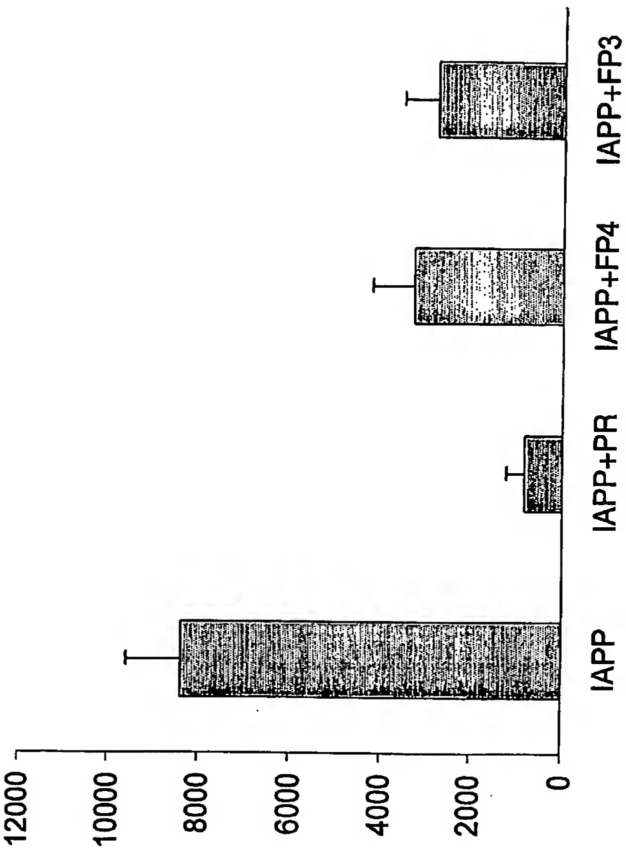


Figure 8a

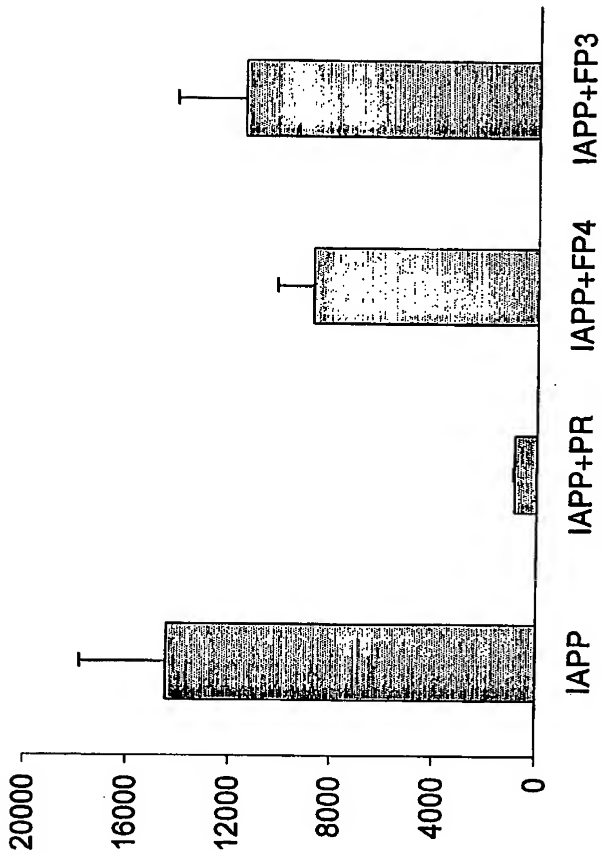


Figure 8b

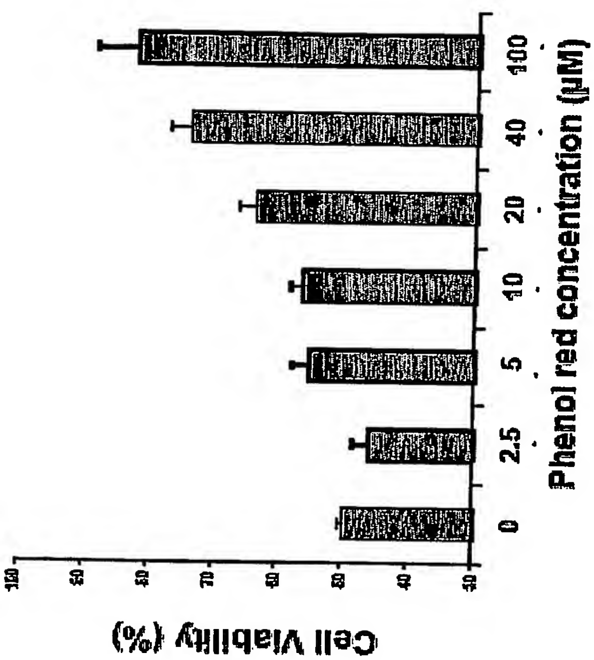
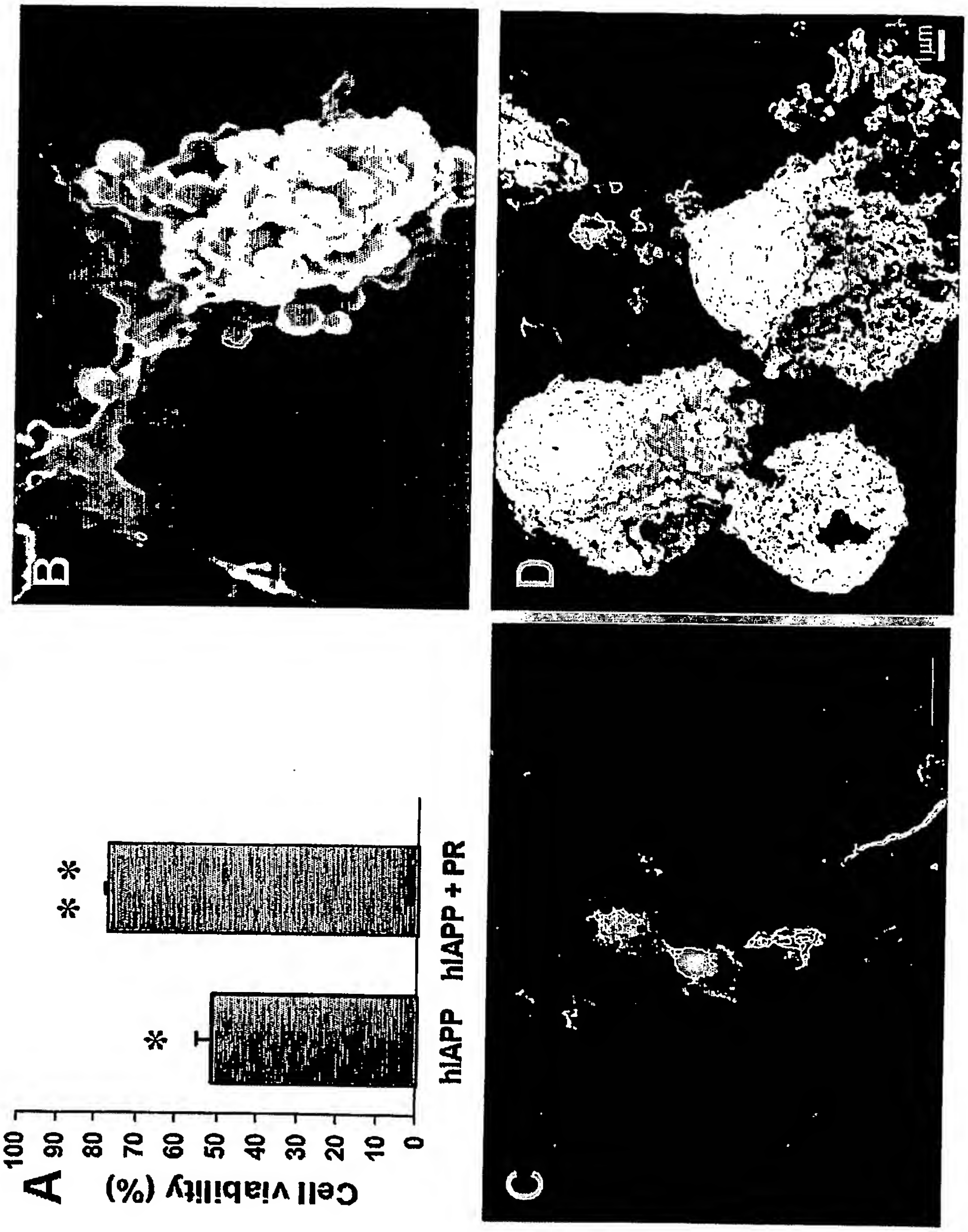
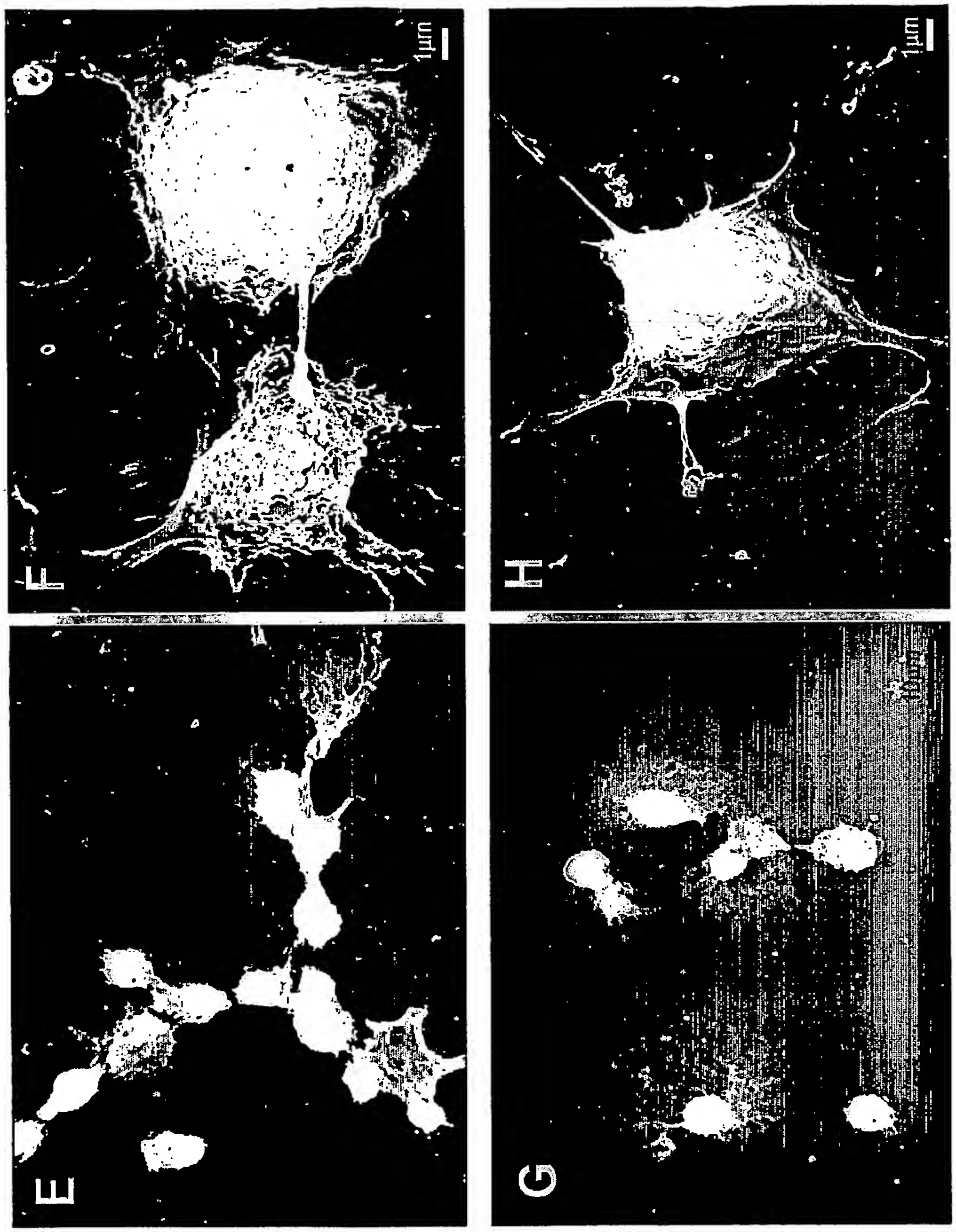

Cell Viability (%)
0
2.5
5
10
20
40
100
Phenol red concentration (µM)

Figure 9



Figures 10a-d



Figures 10e-h

SEQUENCE LISTING

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<110>  Gazit, Ehud
       Porat, Yair

<120>  COMPOSITIONS AND METHODS USING SAME FOR TREATING
       AMYLOID-ASSOCIATED DISEASES

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<160>  3

<170>  PatentIn version 3.2

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<400>  1

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1               5


<210>  2
<211>  10
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<220>
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<400>  2

Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser
1               5                   10


<210>  3
<211>  37
<212>  PRT
<213>  Artificial sequence

<220>
<223>  Synthetic peptide

<400>  3

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
1               5                   10                  15


Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr Asn Val
            20                  25                  30


Gly Ser Asn Thr Tyr
        35
```

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL2004/000890

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/35 A61P25/16 A61P25/28 C07D327/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/49307 A (PROTEOTECH, INC; CASTILLO, GERARDO; SNOW, ALAN, D; CHOI, PAULA, Y) 12 July 2001 (2001-07-12) claims 1,4,9,15	1-9,19, 21-38
X	WO 03/013442 A (PROTEOTECH. INC) 20 February 2003 (2003-02-20) claims 1,3	1-9,19, 21-38
X	PATENT ABSTRACTS OF JAPAN vol. 1998, no. 14, 31 December 1998 (1998-12-31) & JP 10 245342 A (MITSUI NORIN KK), 14 September 1998 (1998-09-14) abstract	1-9,19, 21-38

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 February 2005	23/02/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Beranová, P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL2004/000890

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/49281 A (PROTEO TECH, INC; PROTEOTECH, INC) 12 July 2001 (2001-07-12) claims 1,2	1-9,19, 21-38
X	WO 02/072086 A (CONSORZIO PER GLI STUDI UNIVERSITARI; SUZUKI, HISANORI) 19 September 2002 (2002-09-19) claims 1,2	1-9,19, 21-38
X	US 2002/006954 A1 (HENSLEY KENNETH L ET AL) 17 January 2002 (2002-01-17) claims 1,5	1-9,19, 21-38
X	US 2003/158237 A1 (SARAGOVI URI ET AL) 21 August 2003 (2003-08-21) claims 1,2	1-9,19, 21-38
Y	US 5 332 648 A (KIHARA ET AL) 26 July 1994 (1994-07-26) claims 17,18	41-43
Y	US 4 299 917 A (BERGER ET AL) 10 November 1981 (1981-11-10) claim 1	41-43
Y	LIAO L-L ET AL.: "Tri phenyl methane dyes as inhibitors of reverse transcriptase RNA polymerase and protein synthesis: structure activity relationships" JOURNAL OF MEDICINAL CHEMISTRY, vol. 18, no. 1, 1975, pages 117-120, XP002317348 figure 1D	41-43

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2004/000890

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19 - 29 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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International Application No
PCT/IL2004/000890

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